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DIFFERENTIAL SYSTEMS WITH INTERFACE AND GENERAL BOUNDARY CONDITIONS[1]

By T. J. Pignani[2] and W. M. Whyburn[3]

The first part of this paper studies the system

$$L(Y) = Y' + P(x)Y = Q(x) \tag{1}$$

$$I_r(Y) = A_rY(t_r^+) + B_rY(t_r^-) = C_r, \qquad r = 1, 2, \cdots, m-1, \tag{2}$$

$$H(Y) = AY(a) + BY(b) + \int_a^b F(s)Y(s)\,ds = D, \tag{3}$$

where capital letters denote $(n \times n)$ matrices whose elements in the i^{th} row and j^{th} column are given by the corresponding small letters with subscripts ij, and where $Y' = dY/dx$. Further, A_r, B_r, C_r, A, B and D are matrices of constants, while $P(x)$, $Q(x)$ and $F(x)$ are summable (Lebesgue) matrix functions of the real variable x on the interval $[a, b]: a = t_0^+ \leq x \leq t_m^- = b$. The internal interface points $t_1, t_2, \cdots, t_{m-1}$ are such that $a < t_1 < t_2 < \cdots < t_{m-1} < b$. Symbols $Y(t_r^-)$ and $Y(t_r^+)$ designate the limits of $Y(t)$ as t approaches the point t_r from the left and right, respectively. An Arabic numeral primed enclosed within parentheses (′) designates the homogeneous equation corresponding to the equation of the same unprimed number. The unit matrix $E_k = (e_{ij})$ has $e_{ii} = 1$, $(i = 1, 2, \cdots, k)$, otherwise $e_{ij} = 0$. The zero matrix (with all elements equal to zero) is denoted by the ordinary zero symbol. If A is an $(n \times n)$ matrix, then $|A|$ denotes the n^{th} order determinant of A.

A solution of (1) on an interval $[\alpha, \beta]$ is understood to be a matrix of absolutely continuous elements which satisfies (1) almost everywhere—except for a set of points of measure zero—on $[\alpha, \beta]$. The fundamental existence theorem for equation (1), where the matrices $P(x)$ and $Q(x)$ are summable on $[\alpha, \beta]$, gives a unique solution for which $Y(\gamma) = K$, where $x = \gamma$ is any point of $[\alpha, \beta]$ and K is any given matrix of constants. If $Q(x) \equiv 0$, then Abel's identity,

$$|Y(x)| = |Y(\gamma)| \exp\left[-\int_\gamma^x \sum_1^n p_{ii}(t)\,dt\right],$$

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holds and shows that either $| Y(x) | \equiv 0$ on $[\alpha, \beta]$ or else $| Y(x) |$ does not vanish on this interval. Since K may be chosen so that $| K | \neq 0$, it follows that (1′) has a solution $U(x)$ such that $| U(x) | \neq 0$ on $[\alpha, \beta]$.

A d-solution[4] of (1), (2) on $[a, b]$ is a matrix, $Y(x)$, which is absolutely continuous on $(i - 1, i): t_{i-1} < x < t_i$, where $i = 1, 2, \cdots, m$; satisfies (1) almost everywhere on $[a, b]$; and satisfies condition (2). Frank W. Stallard[5] has investigated system (1), (2), (3) where $F(x) \equiv 0$ on $[a, b]$ and for each r: $C_r = 0$, $| A_r | \neq 0$, $| B_r | \neq 0$. Our study is directed to cases where some of the foregoing conditions fail to hold.

The second part of the paper is concerned with systems (1), (2), (3), with the exception that the number of interface points may be countably infinite. The foregoing definition for a d-solution extends readily to apply to the cases considered.

PART I. FINITE SET OF INTERFACE CONDITIONS

Theorem 1. If at the r^{th} interface point, $1 \leq r \leq m - 1$, A_r and B_r have rank k_r, $0 < k_r \leq n$, and if the non-singular matrices L_{A_r} and R_{A_r}, which satisfy $L_{A_r} A_r R_{A_r} = E_{k_r}$, are such that $L_{A_r} B_r$ has only zeros in its last $(n - k_r)$ rows, then for the equation (2′), where $Y(t_r^-)$ is a known non-singular matrix,

i) there exists a unique non-singular solution for $Y(t_r^+)$ in case $k_r = n$.

ii) when $k_r < n$, there exists an $n(n - k_r)$-parameter family of solutions for $Y(t_r^+)$. Of these, there is at least an $(n - k_r)$-parameter family of solutions each of which is non-singular.

Proof: Multiply equation (2′) on the left by L_{A_r} and on the right by $Y^I(t_r^-)$:

$$E_{k_r} Z = \Lambda,$$

where $Z = R^I_{A_r} Y(t_r^+) Y^I(t_r^-)$ and $\Lambda = L_{A_r} B_r$.

If $k_r = n$, then $Z = \Lambda$, where Λ is of rank n. Hence the solution matrix $Y(t_r^+)$, where $Y(t_r^+) = R_{A_r} \Lambda\, Y(t_r^-)$, is non-singular and unique.

If $k_r < n$, $E_{k_r} Z$ and Λ are matrices with all elements in their last $(n - k_r)$ rows equal to zero while Λ is of rank k_r. Hence Z cannot be of rank less than k_r and elements in k_r of its rows are uniquely determined while the remaining $(n - k_r)$ rows consist of arbitrary elements. The matrix, $Y(t_r^+)$, is given by

$$Y(t_r^+) = R_{A_r} Z Y(t_r^-).$$

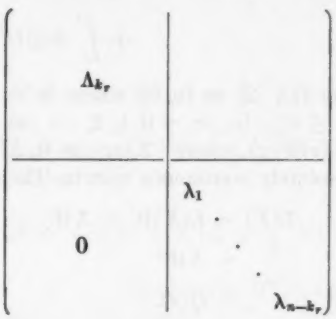
This equation admits an $n(n - k_r)$-parameter family of solutions.

The existence of at least an $(n - k_r)$-parameter family of solutions each of which is non-singular is shown as follows: Multiply Z on the right with a non-singular matrix which makes the k_r-rowed minor in the upper right corner dif-

[4] See Stallard, F. W., "Differential Systems with Interface Conditions," Oak Ridge National Laboratory Publication #1876 (Physics), April 28, 1955. Stallard calls this a Γ-solution.

[5] Loc. cit., pp. 1–34.

ferent from zero. This product is equivalent to Z. Let the elements in the last $(n - k_r)$ rows and first k_r columns have value zero. In the main diagonal of the last $(n - k_r)$ rows and columns assign the parametric values $\lambda_i > 0$. Assign values zero to the remaining elements. Then Z is a matrix of the form:



With the foregoing assignments to the arbitrary elements, Z is a non-singular matrix. From $Y(t_r^+) = R_{A_r} Z Y(t_r^-)$ follows that $Y(t_r^+)$ is non-singular.

If K is a matrix and $x = \gamma$ is any point of $a \leq x \leq t_1$, the fundamental existence theorem for system (1) gives a unique solution on $[a, b]$ of this system which is such that $Y(\gamma) = K$. In particular, this solution determines a unique set of values at $x = t_1$ and these may be used for $Y(t_1^-)$ in the foregoing theorem for the case $r = 1$. For the homogeneous system (1′), non-singularity of K insures non-singularity of $Y(t_1)$. These observations, together with Theorem 1, yield:

Theorem 2. Let the hypotheses of Theorem 1 be valid for each $r = 1, 2, \cdots, m - 1$, where each k_r is a positive integer no greater than n. There exists at least a k-parameter family of d-solutions on $[a, b]$ of system (1′), (2′), $Y(\gamma) = K$, where $k = n^{m-1}k^*$, $k^* = \Pi'^{m-1}_{r=1}(n - k_r)$, and the product omits factors for which $k_r = n$. Furthermore, if K is non-singular, then d-solutions of at least a k^*-parameter family of these are non-singular on $[a, b]$.

Considerations similar to those used in deducing Theorems 1 and 2 establish:

Theorem 3. If $x = \gamma$ is a point of $t_{i-1} < \gamma < t_i$, K is a given constant matrix, the matrices $L_{A_r} B_r$ and $L_{A_r} C_r$ have only zeros in their last $(n - a_r)$ rows, the matrices $L_{B_s} A_s$ and $L_{B_s} C_s$ have only zeros in their last $(n - b_s)$ rows, $(r = 1, \cdots, m - 1)$, $(s = 1, \cdots, i - 1)$, where a_r denotes the rank of A_r and b_r is the rank of B_r, then there exists at least a $|n^{m-1} \prod'^{i-1}_{s=1}(n - b_s) \prod'^{m-1}_{r=i}(n - a_r)|$ family of d-solutions of (1), (2) for which $Y(\gamma) = K$.

For the sequel, the coefficients of (2) are assumed to satisfy the hypotheses of Theorem 3. Let $U(x)$ be a non-singular solution of (1′). Under the assumptions, $X(x) = U(x)M(x)$ is a k^*-parameter family of non-singular d-solutions of (1′), (2′), where $M(x)$ is a non-singular d-solution of (2′). Note that the matrix $M(x)$ is constant throughout each sub-interval $[t_{i-1}^+, t_i^-]$ of $[a, b]$.

Theorem 4. If $X(x) = U(x)M(x)$ is a d-solution of system (1'), (2'), on $[a, b]$, then

$$(4)\quad Y(x) = U(x)M(x)\left[N(s)U^I(s)Y(s) + \sum_{r_s+1}^{r_x} N(t_i^+)U^I(t_i)R_{A_i}L_{A_i}C_i + \int_s^x N(t)U^I(t)Q(t)\,dt\right], \qquad s \le x,$$

is a d-solution of system (1), (2) on $[a, b]$, where $N(x) = M^I(x)$, $a \le x \le b$, $t_{r_s}^+ \le s \le t_{r_s+1}^-$, $t_{r_x}^+ \le x \le t_{r_x+1}^-$ $(r_x, r_s = 0, 1, 2, \cdots, m-1)$.

Proof: Let $Y(x) = X(x)W(x)$, where $|X(x)| \neq 0$, $X^I(x) = V(x)$, and $W(x)$ is an undetermined, absolutely continuous matrix. Then

$$\begin{aligned} L(Y) &= L(X)W + XW' \\ &= XW' \\ &= Q(x). \end{aligned}$$

Hence

$$(5)\qquad W' = V(x)Q(x).$$

Further, condition (2) gives

$$\begin{aligned} I_r(Y) &= A_rY(t_r^+) + B_rY(t_r^-) = C_r \\ &= A_rX(t_r^+)W(t_r^+) + B_rX(t_r^-)W(t_r^-) = C_r. \end{aligned}$$

But

$$I_r(X) = A_rX(t_r^+) + B_rX(t_r^-) = 0,$$

hence a second condition on the matrix, $W(x)$, is

$$(6)\qquad A_rX(t_r^+)[W(t_r^+) - W(t_r^-)] = C_r.$$

Using the hypotheses of Theorem 3 on the matrices A_r and C_r:

$$(7)\qquad W(t_r^+) = W(t_r^-) + T(r, r),$$

where $T(h, r) = \sum_{i=h}^{r} V(t_i^+)R_{A_i}L_{A_i}C_i$, $h \le r$; $T(h, r) = 0$ when $h > r$ $(h, r = 0, 1, 2, \cdots, m-1)$.

One seeks a matrix, $W(x)$, which satisfies (5), (6). A matrix which satisfies (5) almost everywhere on $[a, b]$ is

$$(8)\qquad W(x) = W(s) + \int_s^x V(t)Q(t)\,dt,$$

where s and x are any two points of $[a, b]$. In this expression let $s = a$, $x = t_1^-$, then

$$W(t_1^-) = W(a) + \int_a^{t_1} V(t)Q(t)\,dt.$$

From (7):

$$W(t_1^+) = W(t_1^-) + T(1,1),$$
$$= W(a) + T(1,1) + \int_a^{t_1} V(t)Q(t)\,dt.$$

By induction

$$W(t_r^+) = W(a) + T(1,r) + \int_a^{t_r} V(t)Q(t)\,dt.$$

For any point s in the sub-interval $t_{r_s}^+ \leq s \leq t_{r_s+1}^-$, and when x is in the sub-interval $t_{r_x}^+ \leq x \leq t_{r_x+1}^-$, then

$$W(x) = W(s) + T(r_s+1, r_x) + \int_s^x V(t)Q(t)\,dt \qquad s \leq x,$$

where $r_s, r_x = 0, 1, 2, \cdots, m-1$. This matrix, $W(x)$, satisfies (5) almost everywhere on $[a, b]$ and satisfies (7). The matrix

$$Y(x) = X(x)\left[V(s)Y(s) + T(r_s+1, r_x) + \int_s^x V(t)Q(t)\,dt\right] \qquad s \leq x, \tag{9}$$

satisfies the system (1), (2) on $[a, b]$.

Expression (7) may be rewritten as

$$W(t_r^-) = W(t_r^+) - T(r,r). \tag{10}$$

Formula (8), when $s = b$ and $x = t_{m-1}^+$, becomes

$$W(t_{m-1}^+) = W(b) + \int_b^{t_{m-1}} V(t)Q(t)\,dt.$$

When $r = m-1$ in (10),

$$W(t_{m-1}^-) = W(t_{m-1}^+) - T(m-1, m-1)$$
$$= W(b) - T(m-1, m-1) + \int_b^{t_{m-1}} V(t)Q(t)\,dt.$$

By induction

$$W(t_r^-) = W(b) - T(r, m-1) + \int_b^{t_{m-1}} V(t)Q(t)\,dt,$$

for $r = 1, 2, \cdots, m-1$.

For any point x in $t_{r_x}^+ \leq x \leq t_{r_x+1}^-$, and when s is in the sub-interval $t_{r_s}^+ \leq s \leq t_{r_s+1}^-$, then

$$W(x) = W(s) - T(r_x+1, r_s) - \int_x^s V(t)Q(t)\,dt, \qquad x \leq s,$$

where $r_x, r_s = 0, 1, 2, \cdots, m-1$. This matrix, $W(x)$, also satisfies (5) almost everywhere on $[a, b]$ and satisfies (7). Hence, we have:

Theorem 5. If $X(x) = U(x)M(x)$ is a d-solution of system (1′), (2′) on $[a, b]$, then

$$(11)\quad Y(x) = U(x)M(x)\left[N(s)U^I(s)Y(s) - \sum_{r_s+1}^{r_x} N(t_i^+)U^I(t_i)R_{A_i}L_{A_i}C_i - \int_s^x N(t)U^I(t)Q(t)\,dt\right], \qquad x \le s,$$

is a d-solution of (1), (2) on $[a, b]$, where $N(x) = M^I(x)$, $a \le x \le b$, $t_{r_x}^+ \le x \le t_{r_x+1}^-$, $t_{r_s}^+ \le s \le t_{r_s+1}^-$, $(r_s, r_x = 0, 1, 2, \cdots, m-1)$.

It is noted that when $C_i = 0$ for each $i = 0, 1, 2, \cdots, m-1$, formulae (9) and (11) are equivalent. Further, if all interface conditions on the interval $[a, b]$ reduce to continuity conditions [that is, if in (2), $A_r = -B_r$ and $C_r = 0$ for each $r = 1, 2, \cdots, m-1$], then formulae (9) and (11) reduce to the classical solution formula for (1).

System (1′), (2′), (3′) is compatible if there exists a non-identically-vanishing d-solution on $[a, b]$. A necessary and sufficient condition for this is that $|H(X)| \equiv 0$. When system (1′), (2′), (3′) is assumed incompatible, we show the existence of a k^*-parameter family of d-solutions, $Y(x)$, for (1), (2), (3). This is accomplished by use of three matrices of Green's matrix form.

Pre-multiplication of formula (9) by $F(s)X(s)\,dsV(x)$, followed by integration from a to x; along with pre-multiplication of formula (11) by $F(s)X(s)\,dsV(x)$ and integration from b to x, gives

$$\int_a^x F(s)X(s)\,dsV(x)Y(x) = \int_a^x F(s)Y(s)\,ds + \int_a^x F(s)X(s)T(r_s+1, r_x)\,ds + \int_a^x F(s)X(s)\,ds\int_s^x V(t)Q(t)\,dt,$$

$$\int_b^x F(s)X(s)\,dsV(x)Y(x) = \int_b^x F(s)Y(s)\,ds - \int_b^x F(s)X(s)T(r_x+1, r_s)\,ds + \int_b^x F(s)X(s)\,ds\int_s^x V(t)Q(t)\,dt.$$

Combination of these expressions yields

$$H_2(X)V(x)Y(x) = H_2(Y) + \int_a^b J_2(x, s)\,ds + S_1 - S_2,$$

where

$$H_2(Y) = \int_a^b F(s)Y(s)\,ds,$$

$$J_2(x, s) = \begin{cases} F(s)X(s)T(r_s+1, r_x) & s < x, \\ -F(s)X(s)T(r_x+1, r_s) & s > x, \end{cases}$$

$$S_1 = \int_a^x F(s)X(s)\,ds\int_s^x V(t)Q(t)\,dt,$$

$$S_2 = \int_b^x F(s)X(s)\,ds\int_s^x V(t)Q(t)\,dt.$$

Integration by parts yields

$$S_1 = \int_a^x \int_a^t F(s)X(s)\,ds V(t)Q(t)\,dt, \qquad S_2 = \int_b^x \int_b^t F(s)X(s)\,ds V(t)Q(t)\,dt.$$

Hence

$$H_2(x)V(x)Y(x) = H_2(Y) + \int_a^b J_2(x, s)\,ds + \int_a^b G_2(x, t)Q(t)\,dt, \tag{12}$$

where

$$G_2(x, t) = \begin{cases} \int_a^t F(s)X(s)V(t)\,ds & t < x, \\ \int_b^t F(s)X(s)V(t)\,ds & t > x. \end{cases}$$

Let $s = a$ in formula (9) and pre-multiply by $AX(a)V(x)$. Let $s = b$ in formula (11) and pre-multiply by $BX(b)V(x)$. This gives:

$$AX(a)V(x)Y(x) = AY(a) + AX(a)T(1, r_x) + \int_a^x AX(a)V(t)Q(t)\,dt,$$

$$BX(b)V(x)Y(x) = BY(b) - BX(b)T(r_x + 1, m - 1) + \int_b^x BX(b)V(t)Q(t)\,dt.$$

Addition of these equations gives

$$H_1(X)V(x)Y(x) = H_1(Y) + J_1(x) + \int_a^b G_1(x, t)Q(t)\,dt, \tag{13}$$

where

$$H_1(Y) = AY(a) + BY(b), \qquad J_1(x) = AX(a)T(1, r_x) - BX(b)T(r_x + 1, m - 1),$$

and

$$G_1(x, t) = \begin{cases} AX(a)V(t) & t < x, \\ -BX(b)V(t) & t > x. \end{cases}$$

Let $H(Y) = H_1(Y) + H_2(Y)$ and add (12) to (13):

$$H(X)V(x)Y(x) = H(Y) + J_1(x) + \int_a^b J_2(x, s)\,ds + \int_a^b [G_1(x, t) + G_2(x, t)]Q(t)\,dt.$$

When this expression is multiplied by $X(x)H^I(X)$ on the left and $H(Y)$ is replaced by D, a d-solution of system (1), (2), (3) on $[a, b]$ is given by

$$Y(x) = U(x)M(x)H^I(UM)D + \sum_{i=1}^{m-1} J(x, i)C_i + \int_a^b J^*(x, s)\,ds + \int_a^b G(x, t)Q(t)\,dt, \tag{14}$$

where

$$(15)\qquad J(x,i)=\begin{cases} U(x)M(x)H^I(UM)AU(a)M(a)N(t_i^+)U^I(t_i)R_{A_i}L_{A_i}, & t_i<x,\\ -U(x)M(x)H^I(UM)BU(b)M(b)N(t_i^+)U^I(t_i)R_{A_i}L_{A_i}, & t_i>x,\end{cases}$$

$$(16)\qquad J^*(x,s)=\begin{cases} U(x)M(x)H^I(UM)F(s)U(s)M(s)\displaystyle\sum_{r_s+1}^{r_x}N(t_i^+)U^I(t_i)R_{A_i}L_{A_i}C_i, & s<x,\\ -U(x)M(x)H^I(UM)F(s)U(s)M(s)\displaystyle\sum_{r_x+1}^{r_s}N(t_i^+)U^I(t_i)R_{A_i}L_{A_i}C_i, & s>x,\end{cases}$$

$$(17)\qquad G(x,t)=\begin{cases} U(x)M(x)H^I(UM)\cdot\left[AU(a)M(a)+\displaystyle\int_a^t F(s)U(s)M(s)\,ds\right]N(t)U^I(t), & t<x,\\ -U(x)M(x)H^I(UM)\cdot\left[BU(b)M(b)+\displaystyle\int_t^b F(s)U(s)M(s)\,ds\right]N(t)U^I(t), & t>x.\end{cases}$$

The matrix, $J(x,i)$, has the following properties:

(J.1) $R_{A_i}^I[J(t_i^+,i)-U(t_i)M(t_i^+)N(t_i^-)U^I(t_i)J(t_i^-,i)]\,L_{A_i}^I=E.$

(J.2) $L[J(x,i)]=0$ almost everywhere on $[a,b]$ when i is a fixed index in $1\le i\le m-1$.

(J.3) $I_r[J(x,i)]=0$, when i is a fixed index in $1\le i\le m-1$.

(J.4) If $F(s)\equiv 0$ on $[a,b]$, $H[J(x,i)]=0$, when i is a fixed index in $1\le i\le m-1$.

(J.5) $J(x,i)$ is a member of a k^*-parameter family of such matrices for the differential system.

The matrix $J^*(x,s)$ has the following properties:

(J*.1) $L[J^*(x,\bar{s})]=0$ almost everywhere on $[a,b]$, for fixed $\bar{s}$.

(J*.2) $I_r[J^*(x,\bar{s})]=0$ for fixed s.

(J*.3) $$H\left[\sum_{i=1}^{m-1}J(x,i)C_i+\int_a^b J^*(x,s)\,ds\right]=0.$$

(J*.4) $N(t_\tau^-)U^I(t_\tau)J^*(t_\tau^-,\bar{s})-N(t_{\tau-1}^+)U^I(t_{\tau-1})J^*(t_{\tau-1}^+,\bar{s})=0$ for fixed s.

(J*.5) $$\int_a^b\int_a^b\frac{\partial}{\partial x}[N(x)U^I(x)J^*(x,s)]\,dx\,ds$$
$$=H^I(UM)[H(UM)-AU(a)M(a)-BU(b)M(b)]T(1,m-1).$$

(J*.6) $J^*(x,s)$ is a member of a k^*-parameter family of such matrices of system (1′), (2′).

The matrix $G(x, t)$ is a generalized Green's matrix, and some of its properties are:

(G.1) It is continuous in the rectangle $a \leq x, t \leq b$, except at the interface points $x = t_r$, $t = t_r$ and the line $x = t$, where here it has a finite discontinuity given by $G(x, x^-) - G(x, x^+) = E$, $x \neq t_r$.

(G.2) $L[G(x, \bar{t})] = 0$ almost everywhere on $[a, b]$ for fixed t, $t = \bar{t}$.

(G.3) $I_r[G(x, \bar{t})] = 0$ for fixed t, $t = \bar{t}$.

(G.4) $H[G(x, \bar{t})] = 0$ for fixed t, $t = \bar{t}$.

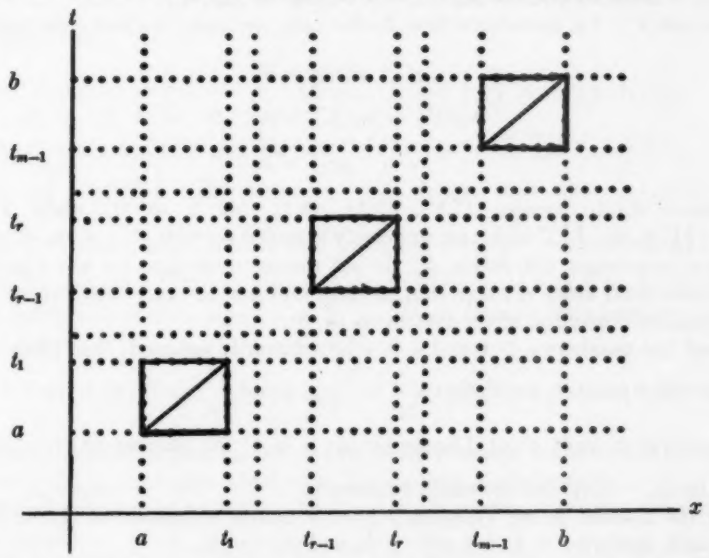
(G.5) $G(x, t)$ is a member of a k^*-parameter family of such Green's matrices for (1'), (2'), (3').

All of the above mentioned properties—except (J*.3) and (G.4)—follow at once from the explicit formulae of the respective matrices. Properties (J*.3) and (G.4) follow by direct substitution into the boundary conditions.

It is noted that the matrix $G(x, t)$ reduces to the classical Green's matrix as developed by W. M. Whyburn[6] if x and t are restricted to a sub-interval $(r-1, r)$. In this case (17) becomes

$$G(x,t) = \begin{cases} U(x)H^I(U)\left[A_{r-1}U(t_{r-1}) + \int_{t_{r-1}}^{t} F(s)U(s)\,ds\right]U^I(t), & t < x, \\ -U(x)H^I(U)\left[B_r U(t_r) + \int_{t}^{t_r} F(s)U(s)\,ds\right]U^I(t), & t > x. \end{cases}$$

This is valid where the interval does not contain interface points which require discontinuity of the solution. It is observed that the following diagram applies, where the classical Green's matrix is obtained in the (solid) squares:



[6] Whyburn, W. M., "Differential Systems with General Boundary Conditions," University of California Publications in Mathematics, N.S., Vol. II No. 1 (1944), pp. 45–61.

If no discontinuities at interface points are involved, these squares become the large square $a \leq t, x \leq b$.

If $F(s) \equiv 0$ in condition (3), we have a two point boundary condition. In this event, $J^*(x, s) \equiv 0$ on $[a, b]$, and the matrix (17) reduces to the ordinary Green's matrix.

For system (1), (2′), (3) formula (14) becomes

$$Y(x) = U(x)M(x)H^I(UM)D + \int_a^b G(x, t)Q(t)\, dt,$$

where $G(x, t)$ is given in (17).

PART II. INTERFACE POINTS FORM A SET OF THE FIRST SPECIES

This section considers the system

$$Y' + P(x)Y = 0 \tag{1′}$$

$$A_tY(t^+) + B_tY(t^-) = C_t, \tag{2}$$

where the points t form a countable subset Γ of $[a, b]$. The set Γ has only two limit points, α and β, neither of which belongs to Γ. Furthermore, only a finite subset of Γ lies outside of the interval $[\alpha, \beta]$: $\alpha \leq x \leq \beta$.

It is noted that only a finite subset of Γ would belong to any interval $[\delta, \eta]$, where $\alpha < \delta < \eta < \beta$. Hence the theorems of Part I are valid on $[\delta, \eta]$. If $x = \gamma$ is a point of $(\alpha, \beta) - \Gamma$, the points of Γ on (α, β) may be so labeled that $\alpha < \cdots < t_{-3} < t_{-2} < t_{-1} < \gamma < t_1 < t_2 < t_3 < \cdots < \beta$. We understand that such labeling is made so that the point $x = \gamma$ belongs to (t_{-1}, t_1).

The case $n = 1$ is considered first. In this case, we study the first order system

$$y' + p(x)y = 0 \tag{1.1′}$$

$$a_ry(t_r^+) + b_ry(t_r^-) = c_r, \quad (r = \pm 1, \pm 2, \cdots). \tag{2.1}$$

$$y(\gamma) = k. \tag{3.1}$$

Theorem 6. In system (1.1′), (2.1), (3.1), let $k \neq 0$, while $1/a_r$, $1/b_{-r}$, $\prod_r^m b_i/a_i$, $\prod_{-r}^{-m} a_i/b_i$, are uniformly bounded for all $0 < r \leq m < \infty$. If $\sum_{-\infty}^{\infty} | c_r |$ converges, a_ib_i and $a_{-i}b_{-i}$ do not change their signs for any i greater than some fixed index r_1, then $\lim_{x \to \alpha^+} y(x)$ and $\lim_{x \to \beta^-} y(x)$ exist, where $y(x)$ is the unique d-solution of the system on (α, β).

Proof: Let numbers $\epsilon_1 > 0$ and $\epsilon_2 > 0$ be arbitrarily assigned; then there exist non-interface points δ, η such that $\alpha < \delta < \eta < \beta$ and $\int_\alpha^\delta | p(t) |\, dt < \log(1 + \epsilon_1)$, $\int_\eta^\beta | p(t) |\, dt < \log(1 + \epsilon_2)$. Denote by $(r_3, r_3 + 1)$ the interval which contains δ and by $(r_4 - 1, r_4)$ the one which contains η.

For the interval $[\delta, \eta]$, Theorem 3 gives a unique d-solution of (1.1′), (2.1), (3.1) such that $y(\gamma) = k$. Let $y(\delta) = k_\delta$ and $y(\eta) = k_\eta$.

For any $r > 0$, the interface condition is

$$y(t_r^+) = f_r + g_r y(t_r^-), \tag{18}$$

where $f_r = c_r/a_r$, $g_r = -b_r/a_r$. The d-solution of (1.1′) which takes on the value $y(s)$ at $x = s$ is

$$y(x) = v(s, x)\, y(s), \tag{19}$$

where $v(s, x) = \exp\left[-\int_s^x p(t)dt\right]$ and $x = s$ is any point of $[a, b]$. Let $v_r = v(t_r, t_{r+1})$, $y_r^- = y(t_r^-)$, $y_r^+ = y(t_r^+)$, $y_0^+ = k$, then from (19),

$$y_r^- = v_{r-1} y_{r-1}^+,$$

and from (18)

$$y_r^+ = f_r + g_r y_r^-,$$

therefore

$$y_r^+ = f_r + g_r v_{r-1} y_{r-1}^+, \qquad (r = 1, 2, \cdots). \tag{20}$$

For any point x in $(m, m + 1)$:

$$\begin{aligned} y(x) &= v(t_m, x) y(t_m^+) \\ &= v^*[f_m + g_m v_{m-1} y_{m-1}^+], \end{aligned}$$

where $v^* = v(t_m, x)$. Further from (20),

$$y(x) = v^*[f_m + g_m v_{m-1} f_{m-1} + g_m v_{m-1} g_{m-1} v_{m-2} y_{m-2}^+].$$

By induction

$$y(x) = v^*\left[f_m + \sum_{r=r_4+1}^{m} \prod_{i=r}^{m} g_i v_{i-1} f_{r-1} + \left(\prod_{r_4+1}^{m} g_i v_{i-1}\right) g_{r_4} v_\eta k_\eta\right], \tag{21}$$

where $v_\eta = v(\eta, t_{r_4})$, $y_\eta^+ = k_\eta$. We note that $\lim_{x \to \beta} v^* = 1$, $\prod_r^m v_{i-1} = v(t_{r-1}, t_m) = \exp\left[-\int_{t_{r-1}}^{t_m} p(t)dt\right]$. The point η was chosen such that $\int_\eta^\beta |p(t)|\, dt < \log(1 + \epsilon_2)$, from which follows that $\prod_r^m v_{i-1} \leq \exp\left[\int_{t_{r-1}}^{\beta}\right] |p(t)|\, dt] < 1 + \epsilon_2$.

By hypothesis, $\prod_r^m g_i$ is bounded uniformly for all r and m. Furthermore, this product is of fixed sign for $m > r_1$. Hence formula (21) expresses $y(x)$ in the form

$$y(x) = \varphi(x) + \sum_{r=r_4}^{m} \varphi_r(x) f_r,$$

where $\lim_{x \to \beta} \varphi(x)$ exists, $|\varphi_r(x)|$ is uniformly bounded for all r and x, and the series $\sum_{r=r_4}^{\infty} f_r$ converges absolutely. Hence $\lim_{x \to \beta^-} y(x)$ exists.

Entirely similar reasoning to the above leads to the conclusion that $\lim_{x \to a^+} y(x)$ exists.

It is noted that any of the many well known conditions for convergence of

infinite products and series may be used to insure the uniform boundedness and convergence required for Theorem 6.

Theorem 7. If i) the conditions of Theorem 6 hold on $[\alpha, \beta]$,

ii) $b_r \neq 0$ at each interface point in $[a, \alpha]$,

and at each such point $a_r \neq 0$ in $[\beta, b]$, then there exists a unique d-solution of (1.1′), (2.1) on $[a, b]$ which satisfies $y(\gamma) = k$ and which is continuous at $x = \alpha$, $x = \beta$.

Proof: Hypothesis (i) contains sufficient conditions for the existence of a unique d-solution of (1.1′), (2.1) on $[\alpha, \beta]$ such that $y(\gamma) = k$.

Define $y(\beta) = \lim_{x \to \beta^-} y(x)$. With $x = \beta$ as the initial point and $y(\beta)$ as the initial condition, Theorem 3 gives a unique d-solution of (1.1′), (2.1), (3.1) on $[\beta, b]$ which satisfies the finite number of interface conditions on $[\beta, b]$.

Similar considerations applied to $[a, \alpha]$, using $\lim_{x \to \alpha^+} y(x)$ as the initial value at $x = \alpha$, gives the desired d-solution of (1.1′), (2.1) on $[a, b]$.

It is noted that the d-solution of Theorem 7 is bounded on $[a, b]$. Thus a form of *stability* is assured for the solution, even though x passes through limit points of the set of interface points as it ranges over $[a, b]$.

The results of Theorem 7 can be extended to certain cases in which Γ' consists of two countable sets, $\{\alpha_r\}$ and $\{\beta_r\}$, where $\cdots \alpha_2 < \alpha_1 < \beta_1 < \beta_2 < \cdots$. In these, a simple sequence of interface points approaches each α_r from the right, and a simple sequence of interface points approaches each β_r from the left. The point $x = \gamma$ is a non-interface point of (α_1, β_1).

The general case $n \geq 1$ is now considered. The set of interface points, Γ, is as described in the introductory paragraph of Part II. Condition (3.1) is now replaced by:

$$Y(\gamma) = K, \tag{4.1}$$

where K is a constant matrix.

Theorem 8. In system (1′), (2), (4.1), let K, A_r, B_{-r} have determinants that are uniformly bounded from zero for all $r = 1, 2, \cdots$. Furthermore, let $\prod_{i=r}^{m} q_{ij}$ and $\prod_{i=r}^{-m} q'_{ij}$ be uniformly bounded for all $0 < r \leq m < \infty$, where q_{ij} is the sum of the absolute values of the elements in the j^{th} column of $-A_i^I B_i$ and q'_{ij} is a similar sum for the matrix $-B_i^I A_i$. For each $r > 0$ let c_r be the sum of the absolute values of the elements of $A_r^I C_r$ and for $r < 0$ let c_r be a similar sum for $B_r^I C_r$. If $\sum_{-\infty}^{\infty} c_r$ converges, then the unique d-solution of (1′), (2) on $[\delta, \eta]$ for which $Y(\gamma) = K$ is such that $y_j(x)$ approaches finite limits as $x \to \beta^-$ and as $x \to \alpha^+$, where $y_j(x) = \sum_{i=1}^{n} |\, y_{ij} \,|$, $(j = 1, 2, \cdots, n)$.

Proof: Write (1′) as

$$y'_{ij} = -\sum_{s=1}^{n} p_{is}(x) y_{sj}, \qquad (i, j = 1, 2, \cdots, n),$$

and note that

$$|\, y'_{ij} \,| \leq h(x) y_j(x),$$

where

$$h(x) = \sum_{i=1}^{n} |p_{ii}(x)|, \qquad y_j(x) = \sum_{i=1}^{n} |y_{ij}|.$$

W. M. Whyburn[7] has shown that $|y'| = ||y|'|$ almost everywhere on $[a, b]$. Hence

$$||y_{ij}|'| \leq h(x)y_j(x).$$

Choose[7] summable functions, $h_{ij}(x)$, $(j = 1, 2, \cdots, n)$, such that $|h_{ij}(x)| \leq |h(x)|$, and

$$|y_{ij}|' = h_{ij}(x)y_j(x),$$

almost everywhere on (α, β). Summation with respect to i yields

$$y_j'(x) = p(x)y_j(x) \tag{22}$$

where

$$y_j' = \sum_{i=1}^{n} |y_{ij}|', \qquad p(x) = \sum_{i=1}^{n} h_{ij}(x), \qquad |p(x)| \leq n|h(x)|$$

and hence $p(x)$ is summable.

From the initial condition:

$$y_j(\gamma) = \sum_{i=1}^{n} |k_{ij}| = k_j. \tag{23}$$

and

$$Y(t_r^+) = F_r + G_rY(t_r^-), \tag{24}$$

where $F_r = A_r^I C_r$, $G_r = -A_r^I B_r$. This generates an interface condition on $y_j(x)$ which is of the form

$$y_j(t_r^+) = f_{r,j} + g_{r,j}y_j(t_r^-), \qquad j = 1, 2, \cdots, n. \tag{25}$$

Condition (24) may be rewritten as

$$y_{ij}(t_r^+) = f_{r,ij} + \sum_{s=1}^{n} g_{r,is}y_{sj}(t_r^-), \qquad (i, j = 1, 2, \cdots, n),$$

where the matrices $F_r = (f_{r,ij}) = A_r^I C_r$, $G_r = (g_{r,ij}) = -A_r^I B_r$; further

$$|y_{ij}(t_r^+)| \leq |f_{r,ij}| + \sum_{s=1}^{n} |g_{r,is}| \cdot |y_{sj}(t_r^-)|.$$

Summation with respect to i yields

$$\sum_{i=1}^{n} |y_{ij}(t_r^+)| \leq \sum_{i=1}^{n} |f_{r,ij}| + \sum_{i=1}^{n} \left[\sum_{s=1}^{n} |g_{r,is}| \cdot |y_{sj}(t_r^-)| \right],$$

[7] See Whyburn, W. M., "Functional Properties of the Solutions of Differential Systems," Transactions of the American Mathematical Society, Vol. XXXII (1930), pp. 502-508.

and

$$y_j(t_\tau^+) \leq \sum_{i=1}^{n} |f_{\tau,ij}| + \sum_{i=1}^{n} \left[\sum_{i=1}^{n} |g_{\tau,ii}| \cdot |y_{ij}(t_\tau^-)| \right],$$

where $y_j = \sum_{i=1}^{n} |y_{ij}|$. Since each $f_{\tau,ij}$ and each $g_{\tau,ii}$ is a constant, there exist constants $f_{\tau,j} \leq \sum_{i=1}^{n} |f_{\tau,ij}|$ and $g_{\tau,j} \leq \sum_{i=1}^{n} |g_{\tau,ij}|$ such that

$$y_j(t_\tau^+) = f_{\tau,j} + g_{\tau,j} y_j(t_\tau^-), \qquad (j = 1, 2, \cdots, n).$$

System (1′), (2) thus leads to system (1.1′), (2.1). The hypotheses of Theorem 6 are met by (22), (25). Hence the unique d-solution of (1′), (2), (4.1) on $[\delta, \eta]$ is such that all $y_j(x)$ approach finite limits as $x \to \beta^-$, $(j = 1, 2, \cdots, n)$.

In a manner similar to that above, it is shown that the unique d-solution of (1′), (2), (4.1) on $[\delta, \eta]$ is such that all $y_j(x)$ approach finite limits as $x \to \alpha^+$, $(j = 1, 2, \cdots, n)$. In this case, the interface condition is

$$Y(t_\tau^-) = F_\tau' + G_\tau' Y(t_\tau^+),$$

where $F_\tau' = B_\tau^I C_\tau$, $G_\tau' = -B_\tau^I A_\tau$. This generates an interface condition on $y_j(x)$ which is of the form

$$y_j(t_\tau^-) = f_{\tau,j}' + g_{\tau,j}' y_j(t_\tau^+), \qquad (j = 1, 2, \cdots, n). \tag{26}$$

Let $\lim_{x \to \beta^-} y_j(x) = \underline{y_j}(\beta)$ and let μ_β be the largest $y_j(\beta)$, where $y_j(x) = \sum_{i=1}^{n} |y_{ij}(x)|$. Then $\overline{\lim}_{x \to \beta} |y_{ij}(x)| \leq \mu_\beta$, and there exists an interval $\beta - \rho \leq x \leq \beta$, $\rho > 0$, such that $|y_{ij}(x)| < \mu_\beta + 1$, $(i, j = 1, 2, \cdots, n)$, for all x on $\beta - \rho \leq x \leq \beta$.

Entirely similar consideration at $x = \alpha$ establishes boundedness of $y_{ij}(x)$ in a neighborhood of $x = \alpha$. We thus have:

Theorem 9. Under the hypotheses of Theorem 8, the d-solution of (1′), (2), (4.1) is stable at the limit points $x = \alpha$ and $x = \beta$ of the set Γ of interface points, where stability is interpreted to mean boundedness for the solution on the interval (α, β).

Under the hypotheses of Theorem 8, the d-solution of (1′), (2), (4.1) on (α, β) may be extended to $[a, b]$ in such a way that

$$y_{ij}(\beta^+) = \overline{\lim}_{x \to \beta^-} y_{ij}(x), \qquad y_{ij}(\alpha^-) = \overline{\lim}_{x \to \alpha^+} y_{ij}(x).$$

In this case, each $y_{ij}(x)$ is upper semi-continuous at $x = \alpha$, $x = \beta$. Similarly, this extension may be made in such a way that the lower limits at $x = \alpha$, $x = \beta$ replace upper limits in the foregoing statement. In such case, lower semi-continuity at $x = \alpha$ and $x = \beta$ results. Also, any combination of choices of upper and lower limits may be used as "initial values" for the solution components at $x = \alpha$ and $x = \beta$. Finally, these "initial values" may be chosen as numbers between the upper and lower limits of the corresponding components.

If any component of $Y(x)$ has a limit as $x \to \beta^-$ or $x \to \alpha^+$, then the d-solution of (1′), (2), (4.1) extends in such a way that it is continuous at this point.

ON THE DISTRIBUTION OF THE JACOBIAN SYMBOLS

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1. *Introduction.* Let p be an odd prime and let RR denote the number of pairs of consecutive quadratic residues in a complete system of residues (mod p). Similarly, let NN denote the number of pairs of consecutive non-residues, RN the number of sequences of one residue and one non-residue, and NR the number of sequences of one non-residue and one residue. In 1896, N. S. Aladov [1] determined these four numbers.

In 1906, E. Jacobsthal [6] proved that if d is relatively prime to p then the sum of all $x^2 + d$ as x ranges over a complete system of residues (mod p) is -1. He used this result not only to compute the four numbers defined above, but also to determine the eight numbers RRR, RRN, RNR, RNN, NRR, NRN, NNR, and NNN, which are similarly defined. He also obtained certain results for sequences of length four. Sequences of length four were later studied by K. Dörge [4] and H. Hopf [5], and sequences of length five and six by H. Davenport [3].

Let m be an odd squarefree number and $m = gh$. Let M^{++} denote the number of pairs of numbers in a complete system of residues (mod m) whose Jacobian symbols are both 1, M^{--} the number of pairs both of whose elements have Jacobian symbols -1, M^{+-} the number of pairs having Jacobian symbols 1 and -1, and M^{-+} the number of pairs having Jacobian symbols -1 and 1. Let similar numbers be defined for g and h. In 1953, A. Brauer [2] proved that if h is prime

$$(1)\qquad \begin{cases} M^{++} = G^{++}H^{++} + G^{+-}H^{+-} + G^{-+}H^{-+} + G^{--}H^{--}, \\ M^{+-} = G^{++}H^{+-} + G^{+-}H^{++} + G^{-+}H^{--} + G^{--}H^{-+}, \\ M^{-+} = G^{++}H^{-+} + G^{+-}H^{--} + G^{-+}H^{++} + G^{--}H^{+-}, \\ M^{--} = G^{++}H^{--} + G^{+-}H^{-+} + G^{-+}H^{+-} + G^{--}H^{++}. \end{cases}$$

Since every Jacobian symbol has value 1, -1, or 0 and since the multiplication of Jacobian symbols can be described by the multiplication of residue classes (mod 3), if we let M^{++}, M^{+-}, M^{-+}, and M^{--} be denoted by M_{11}, M_{12}, M_{21}, and M_{22} respectively, then Brauer's result (1) can be given by one formula,

$$M_{ij} = \sum_{r=1}^{2} \sum_{s=1}^{2} G_{rs} H_{uv}$$

where

$$u \equiv ri,\ v \equiv sj \pmod 3,$$

and in this form one proof is sufficient, rather than a separate proof for each of the four formulas. Using these results, Brauer determined M_{11}, M_{12}, M_{21}, and M_{22} explicitly and also obtained formulas for M_{01}, M_{02}, M_{10}, M_{20}, and M_{00}.

In this paper, the number of triples of consecutive elements in a complete set of residues (mod m) having Jacobian symbols i, j, and k respectively for all i, j, and k (mod 3) will be answered. This number will be denoted by M_{ijk}.

2. *Where i, j, and k are all non-zero.* The following theorem, whose proof is similar to that given by Brauer for sequences of length two, is basic for the determination of M_{ijk}. It will be noted that whereas Brauer required his second factor to be prime, that restriction is here relaxed.

THEOREM 1. Let $m = gh$ be squarefree and relatively prime to 6. Then for i, j, k all non-zero,

$$M_{ijk} = \sum_{r=1}^{2} \sum_{s=1}^{2} \sum_{t=1}^{2} G_{rst} H_{uvw} \tag{2}$$

where

$$u \equiv ir,\ v \equiv js,\ w \equiv kt \pmod 3.$$

Let x be any number for which $x(x+1)(x+2)$ is relatively prime to m. Let r, s, and t denote the residue classes (mod 3) to which the Jacobian symbols (mod g) of x, $x+1$, and $x+2$ respectively belong. Let y denote any number such that the Jacobian symbols (mod h) of y, $y+1$, and $y+2$ belong to the residue classes ir, js, and kt (mod 3) respectively. Then there is exactly one solution (mod m) to the pair of simultaneous congruences

$$z \equiv x \pmod g,\ z \equiv y \pmod h.$$

Since

$$\left(\frac{z}{m}\right) = \left(\frac{z}{g}\right) \cdot \left(\frac{z}{h}\right) \equiv r \cdot ir \equiv \pmod 3,$$

$$\left(\frac{z+1}{m}\right) = \left(\frac{z+1}{g}\right) \cdot \left(\frac{z+1}{h}\right) \equiv s \cdot js \equiv j \pmod 3,$$

and

$$\left(\frac{z+2}{m}\right) = \left(\frac{z+2}{g}\right) \cdot \left(\frac{z+2}{h}\right) \equiv t \cdot kt \equiv k \pmod 3,$$

z, $z+1$, and $z+2$ are consecutive numbers having Jacobian symbols (mod m) congruent to i, j, and k (mod 3) respectively. Hence M_{ijk} is at least as great as the right side of (2).

Conversely, if z is a number such that the Jacobian symbols (mod m) of z, $z+1$, and $z+2$ are i, j, and k (mod 3) respectively, and if their Jacobian symbols (mod g) are denoted by r, s, and t respectively, then their Jacobian symbols (mod h) must be congruent (mod 3) to ir, js, and kt. Hence M_{ijk} is not greater than the right side of (2).

The following results for primes are due to Jacobsthal.

$$P_{ijk} = \tfrac{1}{8}(p-3),\ i,j,k = 1,2 \qquad \text{for } p = 8n+3, \tag{3}$$

$$\begin{cases} P_{111} = P_{211} = P_{221} = P_{222} = \tfrac{1}{8}(p-7), \\ P_{112} = P_{121} = P_{122} = P_{212} = \tfrac{1}{8}(p+1), \end{cases} \qquad \text{for } p = 8n+7, \tag{4}$$

$$\begin{cases} P_{111} = P_{212} = \quad = \tfrac{1}{8}(p-7+2b), \\ P_{112} = P_{121} = P_{211} = P_{222} = \tfrac{1}{8}(p-3-2b), \\ P_{122} = P_{221} = \quad = \tfrac{1}{8}(p+1+2b), \end{cases} \qquad \text{for } p = 8n+5, \tag{5}$$

$$\begin{cases} P_{111} = \quad = \tfrac{1}{8}(p-15+2b), \\ P_{112} = P_{121} = P_{211} = P_{222} = \tfrac{1}{8}(p-3-2b), \\ P_{122} = P_{212} = P_{221} = \quad = \tfrac{1}{8}(p+1+2b), \end{cases} \qquad \text{for } p = 8n+1. \tag{6}$$

The number b occurring in (5) and (6) is defined as the solution of smallest absolute value of the congruence

$$b \equiv \tfrac{1}{2}(-1)^{(p+3)/4}\left(\frac{p-1}{2}\right)!\Big/\left(\frac{p-1}{4}\right)!^2 \pmod{p}. \tag{7}$$

In determining the M_{ijk} we will use Euler's phi-function and Schemmel's [7] generalization $\varphi_n(m)$, where $\varphi_n(m)$ is defined as the number of sets of n consecutive positive integers each less than m and relatively prime to m. It is well known that $\varphi_n(m)$ is multiplicative and that for m squarefree and relatively prime to 6

$$\varphi_3(m) = \prod_{p\mid m}(p-3).$$

Moreover, since every triple of numbers each of whose elements has Jacobian symbol different from zero is counted in one of the M_{ijk}, it is clear that

$$\varphi_3(m) = \sum_{r=1}^{2}\sum_{s=1}^{2}\sum_{t=1}^{2} M_{rst}.$$

For any m divisible by at least one prime of form $4n+1$ there corresponds to each such prime a number b defined by (7). We define $\psi(m)$ to be the product of these b's.

THEOREM 2. If $m = gh$ is squarefree, $(m, 6) = 1$, and h is a prime of form $8n+3$, then

$$M_{ijk} = \tfrac{1}{8}\varphi_3(m), \qquad i,j,k = 1,2. \tag{8}$$

From (2) and (3) it follows that

$$\begin{aligned} M_{ijk} &= \sum_{r=1}^{2}\sum_{s=1}^{2}\sum_{t=1}^{2} G_{rst}\tfrac{1}{8}(h-3) = \tfrac{1}{8}(h-3)\sum_{r=1}^{2}\sum_{s=1}^{2}\sum_{t=1}^{2} G_{rst} \\ &= \tfrac{1}{8}(h-3)\varphi_3(g) = \tfrac{1}{8}\varphi_3(m). \end{aligned}$$

THEOREM 3. If $m = p_1p_2\cdots p_a$ and each p_ρ is distinct and of form $8n+1$, then

$$\begin{cases} M_{111} = \quad = \tfrac{1}{8}[\varphi_3(m) + 2^a\psi(m) + 6(-2)^a], \\ M_{112} = M_{121} = M_{211} = M_{222} = \tfrac{1}{8}[\varphi_3(m) - 2^a\psi(m)], \\ M_{122} = M_{212} = M_{221} = \quad = \tfrac{1}{8}[\varphi_3(m) + 2^a\psi(m) - 2(-2)^a]. \end{cases} \tag{9}$$

For $\alpha = 1$ the theorem is true by (6). Assume it true for $\alpha - 1$ and let $g = p_1 p_2 \cdots p_{\alpha-1}$. Then $m = gp_\alpha$, and

$$(10)\qquad \begin{cases} G_{111} = & = \tfrac{1}{8}[\varphi_3(g) + 2^{\alpha-1}\psi(g) + 6(-2)^{\alpha-1}], \\ G_{112} = G_{121} = G_{211} = G_{222} & = \tfrac{1}{8}[\varphi_3(g) - 2^{\alpha-1}\psi(g)], \\ G_{122} = G_{212} = G_{221} = & = \tfrac{1}{8}[\varphi_3(g) + 2^{\alpha-1}\psi(g) - 2(-2)^{\alpha-1}]. \end{cases}$$

Then by (2), (10), and (6)

$$\begin{aligned} M_{111} &= G_{111}P_{111} + G_{112}P_{112} + G_{121}P_{121} + G_{122}P_{122} \\ &\qquad + G_{211}P_{211} + G_{212}P_{212} + G_{221}P_{221} + G_{222}P_{222} \\ &= G_{111}P_{111} + G_{112}(P_{112} + P_{121} + P_{211} + P_{222}) + G_{122}(P_{122} + P_{212} + P_{221}) \\ &= G_{111}P_{111} + 4G_{112}P_{112} + 3G_{122}P_{122} \\ &= \{\tfrac{1}{64}[\varphi_3(g) + 2^{\alpha-1}\psi(g) + 6(-2)^{\alpha-1}]\cdot[(p-3) + 2b_\alpha - 12] \\ &\quad + \tfrac{1}{16}[\varphi_3(g) - 2^{\alpha-1}\psi(g)]\cdot[(p-3) - 2b_\alpha] \\ &\quad + \tfrac{3}{64}[\varphi_3(g) + 2^{\alpha-1}\psi(g) - 2(-2)^{\alpha-1}]\cdot[(p-3) + 2b_\alpha + 4]\} \\ &= \tfrac{1}{8}(p-3)\varphi_3(g) + \tfrac{1}{4}b_\alpha\cdot 2^{\alpha-1}\psi(g) - \tfrac{3}{8}(-2)^{\alpha-1} \\ &\qquad = \tfrac{1}{8}[\varphi_3(m) + 2^\alpha\psi(m) + 6(-2)^\alpha]. \end{aligned}$$

Similarly

$$\begin{aligned} M_{112} &= G_{111}P_{112} + G_{112}(P_{111} + P_{122} + P_{212} + P_{221}) + G_{122}(P_{121} + P_{211} + P_{222}) \\ &= \{\tfrac{1}{64}[\varphi_3(g) + 2^{\alpha-1}\psi(g) + 6(-2)^{\alpha-1}]\cdot[(p-3) - 2b_\alpha] \\ &\quad + \tfrac{1}{16}[\varphi_3(g) - 2^{\alpha-1}\psi(g)]\cdot[(p-3) + 2b_\alpha] \\ &\quad + \tfrac{3}{64}[\varphi_3(g) + 2^{\alpha-1}\psi(g) - 2(-2)^{\alpha-1}]\cdot[(p-3) - 2b_\alpha]\} \\ &= \tfrac{1}{8}(p-3)\varphi_3(g) - \tfrac{1}{4}b_\alpha\cdot 2^{\alpha-1}\psi(g) = \tfrac{1}{8}[\varphi_3(m) - 2^\alpha\psi(m)]. \end{aligned}$$

In the same manner we obtain

$$M_{121} = M_{211} = M_{222} = M_{112}.$$

Moreover

$$\begin{aligned} M_{122} &= G_{111}P_{122} + G_{112}(P_{121} + P_{112} + P_{222} + P_{211}) + G_{122}(P_{111} + P_{221} + P_{212}) \\ &= \{\tfrac{1}{64}[\varphi_3(g) + 2^{\alpha-1}\psi(g) + 6(-2)^{\alpha-1}]\cdot[(p-3) + 2b_\alpha + 4] \\ &\quad + \tfrac{1}{16}[\varphi_3(g) - 2^{\alpha-1}\psi(g)]\cdot[(p-3) - 2b_\alpha] \\ &\quad + \tfrac{1}{64}[\varphi_3(g) + 2^{\alpha-1}\psi(g) - 2(-2)^{\alpha-1}]\cdot[3(p-3) + 6b_\alpha - 4]\} \\ &= \tfrac{1}{8}(p-3)\varphi_3(g) + \tfrac{1}{4}b_\alpha\cdot 2^{\alpha-1}\psi(g) + \tfrac{1}{2}(-2)^{\alpha-1} \\ &\qquad = \tfrac{1}{8}[\varphi_3(m) + 2^\alpha\psi(m) - 2(-2)^\alpha]. \end{aligned}$$

Finally

$$M_{212} = M_{221} = M_{122}.$$

In the following, empty products are defined as 1.

THEOREM 4. If $m = p_1p_2 \cdots p_\alpha q_1q_2 \cdots q_\beta$ is squarefree and each p_ρ is of form $8n + 1$ and each q_σ is of form $8n + 5$, and $\beta > 0$, then

$$(11)\quad \begin{cases} M_{111} = M_{212} = & = \frac{1}{8}[\varphi_3(m) + 2^{\alpha+\beta}\psi(m) + 2(-2)^{\alpha+\beta}], \\ M_{112} = M_{121} = M_{211} = M_{222} & = \frac{1}{8}[\varphi_3(m) - 2^{\alpha+\beta}\psi(m)], \\ M_{122} = M_{221} = & = \frac{1}{8}[\varphi_3(m) + 2^{\alpha+\beta}\psi(m) - 2(-2)^{\alpha+\beta}]. \end{cases}$$

(I). Suppose $\alpha = 0$. Then the theorem is true for $\beta = 1$ by (5). Assume it true for $\beta - 1$ and let $g = q_1q_2 \cdots q_{\beta-1}$. Then $m = gq_\beta$ and

$$(12)\quad \begin{cases} G_{111} = G_{212} = & = \frac{1}{8}[\varphi_3(g) + 2^{\beta-1}\psi(g) + 2(-2)^{\beta-1}], \\ G_{112} = G_{121} = G_{211} = G_{222} & = \frac{1}{8}[\varphi_3(g) - 2^{\beta-1}\psi(g)], \\ G_{122} = G_{221} = & = \frac{1}{8}[\varphi_3(g) + 2^{\beta-1}\psi(g) - 2(-2)^{\beta-1}], \end{cases}$$

and (11) follows from (2), (12), and (5) by the same method as was used in the proof of Theorem 4.

(II). Let $\alpha > 0$, $g = p_1p_2 \cdots p_\alpha$, and $h = q_1q_2 \cdots q_\beta$. It follows from (11), which has now been established for $\alpha = 0$, that

$$(13)\quad \begin{cases} H_{111} = H_{212} = & = \frac{1}{8}[\varphi_3(h) + 2^{\beta}\psi(h) + 2(-2)^{\beta}], \\ H_{112} = H_{121} = H_{211} = H_{222} & = \frac{1}{8}[\varphi_3(h) - 2^{\beta}\psi(h)], \\ H_{122} = H_{221} = & = \frac{1}{8}[\varphi_3(h) + 2^{\beta}\psi(h) - 2(-2)^{\beta}], \end{cases}$$

and since $m = gh$, (11) now follows from (2), (9), and (13).

THEOREM 5. If $m = p_1p_2 \cdots p_\alpha q_1q_2 \cdots q_\beta$ is squarefree and if each p_ρ is of form $8n + 1$ and each q_σ is of form $8n + 7$ and $\beta > 0$, then for $\beta \equiv 1 \pmod 2$

$$(14)\quad \begin{cases} M_{111} = & = \frac{1}{8}[\varphi_3(m) + (-2)^{\alpha+\beta+2}], \\ M_{112} = M_{121} = M_{122} = M_{211} = M_{221} = M_{222} & = \frac{1}{8}\varphi_3(m), \\ M_{212} = & = \frac{1}{8}[\varphi_3(m) - (-2)^{\alpha+\beta+2}], \end{cases}$$

and for $\beta \equiv 1 \pmod 2$

$$(15)\quad \begin{cases} M_{111} = M_{211} = M_{221} = M_{222} = \frac{1}{8}[\varphi_3(m) - (-2)^{\alpha+\beta+1}], \\ M_{112} = M_{121} = M_{122} = M_{212} = \frac{1}{8}[\varphi_3(m) + (-2)^{\alpha+\beta+1}]. \end{cases}$$

Formulas (14) and (15) will be proved together by a common induction.

(I). Suppose $\alpha = 0$ and $\beta \equiv 0 \pmod 2$. Since for $\alpha = 0$, $\beta = 1$, the theorem is true by (4), assume it true for all integers less than β, and let $g = q_1q_2 \cdots q_{\beta-1}$.

Then $m = gq_\beta$, and

$$(16)\qquad \begin{cases} G_{111} = G_{211} = G_{221} = G_{222} = \frac{1}{8}[\varphi_3(g) - 2^\beta], \\ G_{112} = G_{121} = G_{122} = G_{212} = \frac{1}{8}[\varphi_3(g) + 2^\beta]. \end{cases}$$

Since (14) follows from (2), (4), and (16), we conclude that if the case $\alpha = 0$ is proved for $1, 2, \cdots, \beta - 1$ where β is even, then it holds also for β. In particular, the case $\alpha = 0, \beta = 2$ is proved.

(II). Suppose $\alpha = 0, \beta \equiv 1 \pmod 2$. Since for $\alpha = 0, \beta = 2$ the theorem is true, again assume it true for all integers less than β and let $g = q_1 q_2 \cdots q_{\beta-1}$. Then $m = gq_\beta$, and

$$(17)\qquad \begin{cases} G_{111} = & = \frac{1}{8}[\varphi_3(g) + 2^{\beta+1}], \\ G_{112} = G_{121} = G_{122} = G_{211} = G_{221} = G_{222} = & \frac{1}{8}\varphi_3(g), \\ G_{212} = & = \frac{1}{8}[\varphi_3(g) - 2^{\beta+1}], \end{cases}$$

and (15) follows from (2), (4), and (17). Hence the theorem is true for $\alpha = 0$; that is, if m is a squarefree number each of whose prime factors is of form $8n + 7$, then according as the number β of these prime factors is even or odd, either

$$(18)\qquad \begin{cases} M_{111} = & = \frac{1}{8}[\varphi_3(m) + 2^{\beta+2}], \\ M_{112} = M_{121} = M_{122} = M_{211} = M_{221} = M_{222} = & \frac{1}{8}\varphi_3(m), \\ M_{212} = & = \frac{1}{8}[\varphi_3(m) - 2^{\beta+2}], \end{cases}$$

or

$$(19)\qquad \begin{cases} M_{111} = M_{211} = M_{221} = M_{222} = \frac{1}{8}[\varphi_3(m) - 2^{\beta+1}], \\ M_{112} = M_{121} = M_{122} = M_{212} = \frac{1}{8}[\varphi_3(m) + 2^{\beta+1}]. \end{cases}$$

(III). Suppose $\alpha > 0$. Let $g = p_2 \cdots p_\alpha$ and $h = q_1 q_2 \cdots q_\beta$. Then $m = gh$ and in case $\beta \equiv 0 \pmod 2$, (14) follows from (2), (9), and (18), whereas for $\beta \equiv 1 \pmod 2$, (15) follows from (2), (9), and (19).

THEOREM 6. If $m = p_1 p_2 \cdots p_\alpha q_1 q_2 \cdots q_\beta r_1 r_2 \cdots r_\gamma$ is squarefree and if each p_ρ is of form $8n + 1$, each q_σ of form $8n + 5$, and each r_τ of form $8n + 7$, and if $\beta > 0$ and $\gamma > 0$, then

$$(20)\qquad M_{ijk} = \tfrac{1}{8}\varphi_3(m) \qquad \text{for } i, j, k = 1, 2.$$

Let $g = p_1 p_2 \cdots p_\alpha q_1 q_2 \cdots q_\beta$ and $h = r_1 r_2 \cdots r_\gamma$. Then $m = gh$, and in case $\gamma \equiv 0 \pmod 2$ the theorem follows from (2), (11), and (18), whereas if $\gamma \equiv 1 \pmod 2$, then (20) follows from (2), (11), and (19).

3. *When at least one of i, j, and k is zero.* Brauer proved the following theorem:

If $m = p_1 p_2 \cdots p_r q_1 q_2 \cdots q_s$ where the p_ρ are different primes of form $4n + 1$ and the q_σ different primes of form $4n + 3$, then

$$
(21)\qquad \begin{cases} M_{11} = M_{22} = M_{21} = M_{12} + 1 = \tfrac{1}{4}[\varphi_2(m) + 1] \\ \qquad\qquad \text{if } r \equiv s \equiv 1 \pmod 2, \\ M_{22} = M_{12} = M_{21} = M_{11} + 1 = \tfrac{1}{4}[\varphi_2(m) + 1] \\ \qquad\qquad \text{if } r \equiv 1, s \equiv 0 \pmod 2, \\ M_{11} = M_{22} = M_{21} = M_{12} - 1 = \tfrac{1}{4}[\varphi_2(m) - 1] \\ \qquad\qquad \text{if } r \equiv 0, s \equiv 1 \pmod 2, \\ M_{22} = M_{12} = M_{21} = M_{11} - 1 = \tfrac{1}{4}[\varphi_2(m) - 1] \\ \qquad\qquad \text{if } r \equiv s \equiv 0 \pmod 2. \end{cases}
$$

These results can be used together with (8), (9), (11), (14), (15), and (20) to obtain the following eight numbers immediately, since for each pair of numbers x and $x + 1$ having Jacobian symbols congruent to i and j respectively (mod 3), the Jacobian symbol of $x + 2$ has to be congruent either to 0, 1, or 2 (mod 3). Therefore

$$
(22)\qquad \begin{cases} M_{110} = M_{11} - (M_{111} + M_{112}), & M_{011} = M_{11} - (M_{111} + M_{211}), \\ M_{120} = M_{12} - (M_{121} + M_{122}), & M_{012} = M_{12} - (M_{112} + M_{212}), \\ M_{210} = M_{21} - (M_{211} + M_{212}), & M_{021} = M_{21} - (M_{121} + M_{221}), \\ M_{220} = M_{22} - (M_{221} + M_{222}), & M_{022} = M_{22} - (M_{122} + M_{222}). \end{cases}
$$

The following theorem is a generalization of a result due to Brauer.

THEOREM 7. Let M_{ij}^{d} denote the number of elements x in a complete system of residues (mod m) for which $(x/m) \equiv i \pmod 3$ and $[(x + d)/m] \equiv j \pmod 3$. Then

$$
M_{ij}^{d} = M_{ij} \quad \text{if} \quad (d/m) = 1, \quad \text{and} \quad M_{ij}^{d} = M_{kl} \quad \text{if} \quad (d/m) = -1,
$$

where

$$
k \equiv -i, \qquad l \equiv -j \pmod 3.
$$

(I). Suppose $(d/m) = 1$. Let x be a number such that

$$
\left(\frac{x}{m}\right) \equiv i, \qquad \left(\frac{x + d}{m}\right) \equiv j \pmod 3.
$$

and let

$$
dy \equiv 1 \pmod m.
$$

Then $(y/m) = 1$, and

$$
\left(\frac{xy}{m}\right) = \left(\frac{x}{m}\right) \equiv i \pmod 3,
$$

$$
\left(\frac{xy + 1}{m}\right) = \left(\frac{xy + dy}{m}\right) \equiv j \pmod 3
$$

Hence M_{ij}^{d} is not greater than M_{ij}.

Conversely, let x be a number such that

$$\left(\frac{x}{m}\right) \equiv i, \qquad \left(\frac{x+1}{m}\right) \equiv j \pmod 3.$$

Then

$$\left(\frac{dx}{m}\right) = \left(\frac{x}{m}\right) \equiv i \pmod 3, \qquad \left(\frac{dx+d}{m}\right) = \left(\frac{x+1}{m}\right) \equiv j \pmod 3.$$

Hence M_{ij}^{d} is as great as M_{ij}.

(II). Suppose $(d/m) = -1$. Again let x be a number such that

$$\left(\frac{x}{m}\right) \equiv i, \qquad \left(\frac{x+d}{m}\right) \equiv j \pmod 3,$$

and let

$$dy \equiv 1 \pmod m.$$

Then $(y/m) = -1$, and

$$\left(\frac{xy}{m}\right) = -\left(\frac{x}{m}\right) = -i \pmod 3,$$

$$\left(\frac{xy+1}{m}\right) = \left(\frac{xy+dy}{m}\right) = -\left(\frac{x+d}{m}\right) \equiv -j \pmod 3.$$

Hence M_{ij}^{d} is not greater than M_{kl}.

Conversely, let x be a number such that

$$\left(\frac{x}{m}\right) \equiv k, \qquad \left(\frac{x+1}{m}\right) = l \pmod 3.$$

Then

$$\left(\frac{dx}{m}\right) \equiv i, \qquad \left(\frac{dx+d}{m}\right) \equiv j \pmod 3.$$

Hence M_{ij}^{d} is exactly M_{kl}.

Letting $d = 2$ in Theorem 7, it follows that if m contains an even number of prime factors of form $8n + 3$, then

$$(23) \qquad \begin{cases} M_{101} = M_{11} - (M_{111} + M_{121}), \\ M_{102} = M_{12} - (M_{112} + M_{122}), \\ M_{201} = M_{21} - (M_{211} + M_{221}), \\ M_{202} = M_{22} - (M_{212} + M_{222}), \end{cases}$$

whereas if m contains an odd number of such prime factors, then

$$(24)\qquad \begin{cases} M_{101} = M_{22} - (M_{111} + M_{121}), \\ M_{102} = M_{21} - (M_{112} + M_{122}), \\ M_{201} = M_{12} - (M_{211} + M_{221}), \\ M_{202} = M_{11} - (M_{212} + M_{222}). \end{cases}$$

The following theorem gives Brauer's results for the numbers M_{01}, M_{02}, M_{10}, M_{20}, and M_{00}.

If $m = p_1p_2 \cdots p_rq_1q_2 \cdots q_s$ where the p_ρ are different primes of form $4n + 1$ and the q_σ different primes of form $4n + 3$, then

$$(25)\qquad \begin{cases} M_{01} = \tfrac{1}{2}[\varphi(m) - \varphi_2(m) + 1] = M_{02} + 1 & \text{if } r \not\equiv s \pmod 2, \\ M_{01} = \tfrac{1}{2}[\varphi(m) - \varphi_2(m) - 1] = M_{02} - 1 & \text{if } r \equiv s \pmod 2, \\ M_{10} = \tfrac{1}{2}[\varphi(m) - \varphi_2(m) + 1] = M_{20} + 1 & \text{if } r \equiv 1 \pmod 2, \\ M_{10} = \tfrac{1}{2}[\varphi(m) - \varphi_2(m) - 1] = M_{20} - 1 & \text{if } r \equiv 0 \pmod 2, \\ M_{00} = m - 2\varphi(m) + \varphi_2(m). \end{cases}$$

Using (22), (23), (24), and (25), the following numbers can be determined immediately.

$$M_{100} = M_{10} - (M_{101} + M_{102}),$$

$$M_{200} = M_{20} - (M_{201} + M_{202}),$$

$$M_{010} = M_{10} - (M_{110} + M_{210}) = M_{01} - (M_{011} + M_{012}),$$

$$M_{020} = M_{20} - (M_{120} + M_{220}) = M_{02} - (M_{021} + M_{022}),$$

$$M_{001} = M_{01} - (M_{101} + M_{201}),$$

$$M_{002} = M_{02} - (M_{102} + M_{202}),$$

$$M_{000} = M_{00} - (M_{001} + M_{002}) = M_{00} - (M_{100} + M_{200}).$$

Since

$$\sum_{i=1}^{2}\sum_{j=1}^{2} M_{ij} = \varphi_2(m) \quad \text{and} \quad \sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{k=1}^{2} M_{ijk} = \varphi_3(m),$$

this last relation can be written

$$M_{000} = m - 3\varphi(m) + 3\varphi_2(m) - \varphi_3(m).$$

REFERENCES

1. ALADOV, N. S. Sur la distribution des résidus et non-résidus quadratiques d'un nombre premier dans la suite 1, 2, . . . , p-1. Mat. Sbornik **18**: 61–75. 1896.
2. BRAUER, A. On the distribution of the Jacobian symbols. Math. Zeitschr. **58**: 226–231. 1953.
3. DAVENPORT, H. On the distribution of quadratic residues (mod p). Jour. London Math. Soc. **6**: 49–54. 1931. *Ibid.* **8**: 46–52. 1933.

4. DÖRGE, K. Zur Verteilung der quadratischen Reste. Jber. dtsch. Math.-Verein. **38:** 41–49. 1929.
5. HOPF, H. Über die Verteilung der quadratischen Reste. Math. Zeitschr. **32:** 222–231. 1930.
6. JACOBSTHAL, E. Anwendung einer Formel aus der Theorie der quadratischen Reste. Dissertation, Univ. Berlin, 1906.
7. SCHEMMEL, V. Ueber relative Primzahlen. Jour. reine angew. Math. **70:** 191–192. 1869.

A NEW SUBSPECIES OF THE LONGTAIL SHREW (*SOREX DISPAR* BATCHELDER) FROM THE SOUTHERN APPALACHIAN MOUNTAINS

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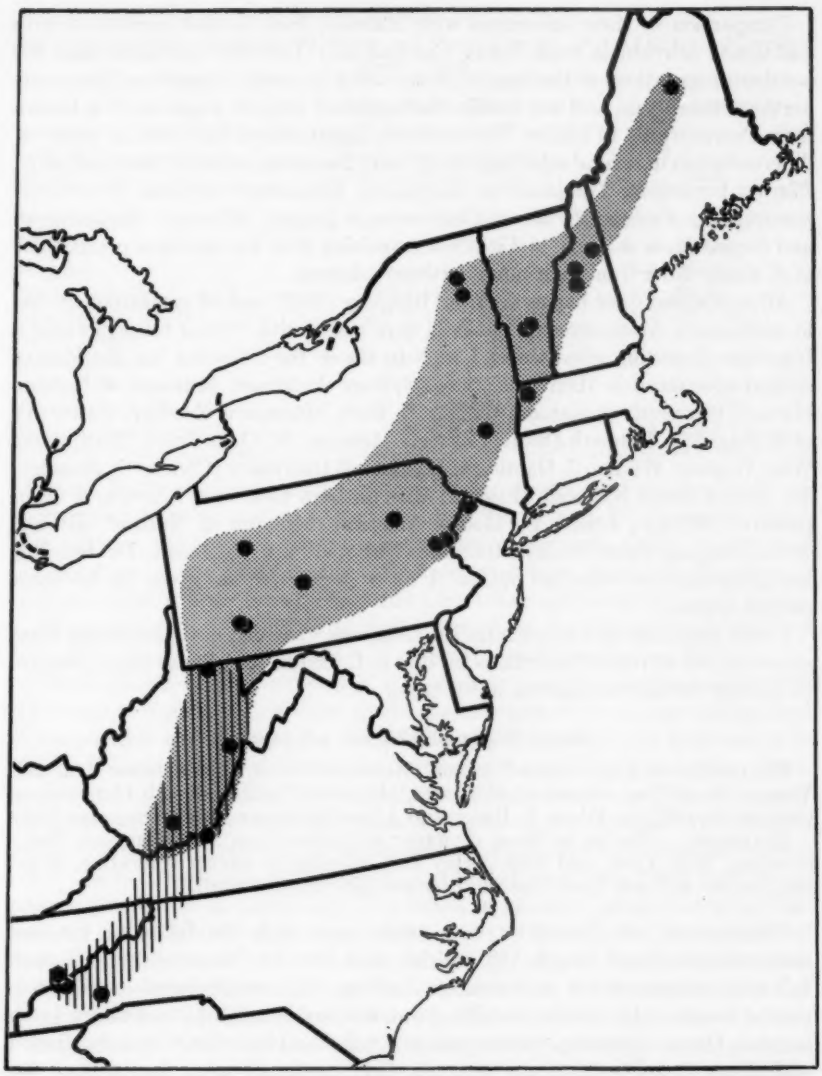
At the time of Jackson's revision (1928) of the American long-tailed shrews, fifteen specimens of the rare *Sorex dispar* were available for study. Of these, three were from Massachusetts, ten from New York, one from Pennsylvania, and one from West Virginia. Since the time of Batchelder's description of this shrew (as *Sorex macrurus*) in 1896, only a very few specimens had been collected. Subsequent records of occurrence have been published by many authors to add to the known northern distribution of this species. Records have been published for Vermont (Osgood, 1935, 1938), New Hampshire (Mather, 1933; Lincoln, 1935; Preble, 1937), New York (Harper, 1929; Tate, 1935), Maryland (Mansueti and Flyger, 1952), Maine (Starrett, 1954), and West Virginia (Kellogg, 1937; Wilson and Friedel, 1942; McKeever, 1951). Conaway and Pfitzer (1952) first reported *S. dispar* from the southern Blue Ridge mountains; they mentioned specimens of this species taken from one locality in North Carolina, and from three localities in Tennessee. A previously unrecorded individual had been taken on Clingman's Dome, North Carolina. These records for Tennessee and North Carolina extended the known range of *S. dispar* some 200 miles southward from the formerly southernmost record in West Virginia.

In the autumn of 1955, two additional specimens of *S. dispar* were taken by Edwin L. Blitch and myself near Wagon Road Gap, Haywood County, North Carolina, at an elevation of 4525 feet. Wagon Road Gap lies immediately to the southwest of Mt. Pisgah, which nearby rises to an elevation of 5749 feet. These specimens were taken from a moist and shaded talus slope immediately above the Pisgah Motor Road, in situations quite comparable to those noted for the same species in Pennsylvania by Richmond and Grimm (1950). The first individual was trapped on October 16, 1955; the previous afternoon had been extremely cold and there had been a steady snowfall for approximately 1½ hours in the midafternoon. The second specimen was taken on October 30, 1955; on the previous day the weather had again been cool, but without any precipitation.



MAP 1. Eastern United States, showing the distribution of *Sorex dispar*. Solid circles represent localities from which specimens of *S. dispar* have been examined; locality dots have been condensed in several areas for clarity. The stippled area represents the range of *S. d. dispar*, and the area with vertical lines represents the range of *S. d. blitchi*. The region of intergradation is shown by the overlap of the two types of shading.

Comparison of these specimens with material from farther north and with additional individuals from North Carolina and Tennessee indicates that the southern populations of the longtail shrew differ in several characters from more northern specimens, and are readily distinguished from *S. dispar* as it is known from Pennsylvania to Maine. The southern Appalachians have been a center of differentiation in several other species of small mammals, notably *Sorex palustris*, *Blarina brevicauda*, *Tamiasciurus hudsonicus*, *Glaucomys sabrinus*, *Peromyscus maniculatus*, *Neotoma floridana*, *Clethrionomys gapperi*, *Microtus chrotorrhinus*, and *Napaeozapus insignis*, and it is not surprising that the southern populations of *S. dispar* differ from their more northern relatives.

All capitalized color names are from Ridgway (1912) and all measurements are in millimeters. Measurements of skulls were taken with vernier calipers under a binocular dissecting microscope. I wish to thank the following for the loan of critical specimens in their collections: Sydney Anderson, Museum of Natural History, University of Kansas; William H. Burt, Museum of Zoology, University of Michigan; J. Kenneth Doutt, Carnegie Museum; W. Gene Frum, Huntington, West Virginia; William J. Hamilton, Jr., Cornell University; Charles O. Handley, Jr., United States National Museum; Miss Barbara Lawrence, Museum of Comparative Zoology; Joseph C. Moore, American Museum of Natural History; Arthur Stupka, Great Smoky Mountains National Park, Tennessee. Dr. Handley has generously curtailed his own work on *S. dispar* and has given me his notes on this species.

I wish especially to acknowledge the assistance extended me in collecting these shrews in the southern mountains by Edwin L. Blitch, and I take great pleasure in naming the new subspecies in his honor.

Sorex dispar blitchi, new subspecies.

HOLOTYPE.—United States National Museum *301993*, a male, taken 2 mi. NE Wagon Road Gap, elevation 4525 feet, Haywood County, North Carolina, on October 30, 1955, by Edwin L. Blitch and Albert Schwartz. Original number *3525*.

DIAGNOSIS.—Similar to *Sorex d. dispar* as known from New England, Pennsylvania, New York, and New Jersey, but differing in darker coloration, larger size, longer tail and hind foot, and longer and broader skull.

DESCRIPTION OF HOLOTYPE.—An adult male with the following external measurements: total length 130, tail 65, hind foot 16. Measurements of skull (all skull measurements as taken by Jackson, 13): condylobasal length 18.4; palatal length, 7.15; cranial breadth 3.6; maxillary breadth 4.6; maxillary tooth row 6.5. Dorsal coloration (winter pelage): individual hairs black basally, tipped with Deep Grayish Olive, the over-all effect produced being darker than the dorsal coloration of *S. d. dispar*. Ventral coloration: individual hairs black basally, tipped with white, giving a silvery effect. Dorsal and ventral colorations grade gradually along sides. Chin and throat fur gray basally, and tipped with white. Tail indistinctly bicolor, darker above. Front and hind feet pale gray; ankles dark gray, similar to dorsal color.

VARIATION.—Aside from the holotype, there are ten specimens of *S. d. blitchi*

TABLE I

External and cranial measurements (means and observed ranges, in mm.) of two subspecies of Sorex dispar.

	Sorex d. dispar	*Sorex d. blitchi*
Number	18 ♂, 14 ♀, 2 unsexed	6 ♂, 5 ♀
Locality	New England, Penn, N. Y., N. J.	N. C., Tenn.
Total length	117.1 (103–139)	130.7 (123–135)
Tail	55.6 (48–61)	64.0 (62–66)
Hind foot	14.1 (13–15)	15.5 (15–16)
Head and body	61.5 (48–78)	66.7 (60–72)
Condylobasal length	17.7 (17.4–18.2)	18.3 (18.1–18.5)
Palatal length	7.0 (6.5–7.3)	7.4 (7.1–7.9)
Cranial breadth	8.0 (7.5–8.5)	8.3 (8.0–8.7)
Interorbital breadth	3.3 (3.1–3.7)	3.6 (3.5–3.8)
Maxillary breadth	4.3 (3.5–4.6)	4.7 (4.4–4.9)
Maxillary tooth row	6.3 (6.0–6.5)	6.5 (6.3–6.7)

at hand. Eight of these are in the deep winter pelage, and another is in the shorter, less dense summer fur. One individual (taken April 29) shows the transition between the pelages. Winter-taken specimens are very similar to the type in coloration; all are dark dorsally and silvery ventrally. The single specimen in summer pelage is more brown (Brownish Olive) than specimens in winter pelage.

All specimens of *S. d. blitchi* are characterized by the larger size, larger hind foot, and longer tail. For cranial and external measurements, see Table I.

COMPARISONS.—Thirty skins of *S. d. dispar* are available for comparison with specimens of *S. d. blitchi*. Of these specimens, nineteen are in summer pelage, and the remainder are in winter fur. The winter pelage is deep and comparable to the winter pelage in *S. d. blitchi* in texture and depth. The dorsal fur is black basally and tipped with Dark Olive Buff. The dorsal coloration is thus, in over-all effect, less dark (more brownish) than winter-taken *S. d. blitchi*. The summer pelage is not so long nor so deep as the winter pelage, but is comparable to it in color; the hairs are again black basally and tipped with Deep Olive Buff. Comparison between *S. d. dispar* and *S. d. blitchi* in summer pelage is difficult, because of inadequate summer-taken material of the latter form. On the basis of the single specimen of *S. d. blitchi* in summer pelage, the southern subspecies appears to be somewhat darker than the northern race, but this difference is not striking and must await further confirmation from additional specimens.

Inspection of Table I shows that *S. d. blitchi* averages larger in all external and cranial measurements taken. The most striking difference lies in the length of tail; on the basis of this character, specimens can be allocated to one or the other of the two subspecies, and there is no overlap in length of tail. The over-all size of the two races is likewise striking, although occasional specimens of *S. d. dispar* equal or exceed *S. d. blitchi* in total length. Cranially, *S. d. blitchi* averages consistently larger in all measurements, and the amount of overlap between the two subspecies is relatively slight.

Although there are but five specimens of *S. dispar* available from Virginia and West Virginia, these animals are considered as intergrades between *S. d. dispar* and *S. d. blitchi*. In pelage color (three in summer pelage, two in winter) all specimens more closely resemble *S. d. dispar*. The tail lengths on the five individuals are 53, 58, 58, 59, and 62; the individual with the longest tail is from West Virginia. Cranially the series appears intermediate between the two subspecies. The condylobasal lengths of the three measureable skulls are 18.4, 17.9, and 17.9; the higher of these measurements is within the known range of variation in *S. d. blitchi*, while the lower are near the upper extreme in *S. d. dispar*. It is apparent that intergradation between the two subspecies occurs in the central portion of the Appalachian mountains.

REMARKS.—Jackson (p. 90) noted that of the fifteen specimens examined by him, only a single skin (a male, from Hunter Mountain, N. Y.) was in the process of molting. This specimen had been taken on September 4, 1896; the winter fur was beginning to appear beneath the worn summer pelage on the rump. The accumulated material at hand allows for some additional information on the molt of *Sorex dispar*. In the north, the winter pelage appears in September; specimens taken between September 29 and April 28 show the winter fur. The summer pelage is displayed by individuals taken between April 28 and September 30. However, not all shrews in a local population achieve the molt at precisely the same time; of three specimens from Jefferson County, Pennsylvania, taken on April 28, 1948, two are in winter pelage and show no signs of molt, whereas the third is in complete summer pelage. In the south, specimens from Tennessee and North Carolina show winter pelage between October 16 and April 27, although a single specimen taken October 2 still retains the summer pelage. These dates are roughly comparable to those for molt in the north. One specimen, taken April 28, 1952, on the West Prong of the Little Pigeon River, Sevier County, Tennessee, is in the process of molting. The summer fur occurs in a dorsal band from the level of the pinnae to the posterior third of the back, and the winter fur is still retained on the head and nape, as well as upon the rump. Jackson (p. 18) noted that members of the genus *Sorex* usually molt, in the spring, from the nape and crown posteriorly, the rump being the last portion to receive the new fur. However, he also mentioned that members of the subgenera *Neosorex* and *Atophyrax* frequently have the new hair first appearing on the middle of the dorsum. This condition he considered exceptional in *Sorex*. Additional specimens of *S. dispar* will be required to demonstrate what is the characteristic spring and fall molting pattern in this species.

The specimens of *S. d. blitchi* presently available indicate that this subspecies occurs at high elevations in the southern Appalachians. Conaway and Pfitzer (p. 107) recorded the taking of individuals in North Carolina and Tennessee at elevations from 3700 feet (West Prong, Little Pigeon River) to 6642 feet (Clingman's Dome); the two specimens from Mt. Pisgah are from an elevation intermediate between those recorded by these authors. The habitat of the type and topotype of *S. d. blitchi* is a natural talus slope, immediately above the Pisgah Motor Road. This slope is well shaded with deciduous trees and the rocks

are partially moss-covered with extensive fractures and deep crevices. Traps for small mammals were set as deeply as possible within these crevices, and, along with *S. d. blitchi*, were taken specimens of *Peromyscus m. nubiterrae*, *Peromyscus l. leucopus*, and *Microtus c. carolinensis*. A single *Tamias s. striatus* was also observed on the rock slide. Almost certainly, specimens of *Sorex dispar* will ultimately be secured in Georgia, and possibly South Carolina, if trapping is carried on at high elevations in the macro- and microhabitat wherein this rare shrew occurs. That its occurrence is not limited to the Great Smoky Mountains also serves to demonstrate that it is probably far more widespread in the southern mountains than the present meager records indicate.

SPECIMENS EXAMINED. *Sorex d. dispar*: MAINE: *Piscataquis Co.*, Baxter State Park, South Branch Pond, 1000 ft., 1. NEW HAMPSHIRE: *Coos Co.*, Mt. Washington, 3560–3650 ft., 3; *Grafton Co.*, Kinsman Notch, Lost River, 1900 ft., 1; Liberty Spring, Franconia Ridge, 3900 ft., 1. VERMONT: *Rutland Co.*, Sherburne, 2500 ft., 1; Mendon, 1400 ft., 3. NEW YORK: *Essex Co.*, Mt. Marcy, summit, 5344 ft., 1; Ray Brook, 1630 ft., 1; Beede Brook, 1700 ft., 1; Whiteface Mt., 3300 ft., 1; *Greene Co.*, Catskill Mts., Hunter Mt., 7. MASSACHUSETTS: *Berkshire Co.*, Adams (Mt. Greylock, 3400 ft.), 1. NEW JERSEY, *Sussex Co.*, Stillwater Township, 1. PENNSYLVANIA: *Luzerne Co.*, 7½ mi. WNW Sweet Valley, 1; 8 mi. WNW Sweet Valley, 1; *Jefferson Co.*, 5½ mi. NE Sigel, 3; *Northampton Co.*, ½ mi. NW Windgap, 1200 ft., 1; 1 mi. N. Danielsville, 1200 ft., 1; *Huntingdon Co.*, 2 mi. NE Spruce Creek, 900 ft., 1; *Westmoreland Co.*, 4 mi. SE Latrobe, 1060 ft., 1; 4 mi. SSE Rector, 2100 ft., 1; 2 mi. SSE Rector, 1600 ft., 1.

Intergrades between *S. d. dispar* and *S. d. blitchi*: WEST VIRGINIA: *Monongalia Co.*, ½ mi. SE Dellslow, 1200 ft., 2; *Randolph Co.*, 5 mi. NNW Durbin, 4350 ft., 1; *Raleigh Co.*, Winding Gulf, SW of Pemberton, 1. VIRGINIA: *Giles Co.*, 4.2 mi. NNE Mt. Lake, Castle Rock Big Mt., 4100 ft., 1.

Sorex d. blitchi: TENNESSEE: *Sevier Co.*, Walker Prong, Great Smoky Mountains National Park, 4400–4500 ft., 2; bet. Highway 71 and West Prong, Little Pigeon River, 3400 ft., 2. NORTH CAROLINA: *Swain Co.*, Clingman's Dome, 6400–6642 ft., 4; talus slope above Highway 107, 4400 ft., 1; *Haywood Co.*, 2 mi. NE Wagon Road Gap, 4525 ft., 2.

LITERATURE CITED

CONAWAY, CLINTON H., AND DONALD W. PFITZER. 1952. *Sorex palustris* and *Sorex dispar* from the Great Smoky Mountains National Park. Jour. Mamm. **33:** 106–108.

HARPER, FRANCIS. 1929. Notes on the mammals of the Adirondacks. N. Y. State Mus. Handbook, No. 8: 51–118.

JACKSON, H. H. T. 1928. A taxonomic review of the American long-tailed shrews (genera *Sorex* and *Microsorex*). North Amer. Fauna, No. 51: vi + 238 pp.

KELLOGG, REMINGTON. 1937. Annotated list of West Virginia mammals. Proc. U. S. Nat. Mus. **84:** 443–479.

LINCOLN, ALEXANDER, JR. 1935. *Sorex dispar* in New Hampshire. Jour. Mamm. **16:** 223.

MATHER, DEANE W. 1933. Gray long-tailed shrew from New Hampshire. Jour. Mamm. **14:** 70.

MCKEEVER, STURGIS. 1951. A survey of West Virginia mammals. Conserv. Comm. West Va. v + 126 pp. (mimeo.).

MANSUETI, ROMEO, AND VAGN F. FLYGER. 1952. Long-tailed shrew (*Sorex dispar*) in Maryland. Jour. Mamm. **33:** 250.

OSGOOD, F. L. 1935. Four Vermont records of the big-tailed shrew. Jour. Mamm. **16:** 146.

———. 1938. The mammals of Vermont. Jour. Mamm. **19:** 435–441.

PREBLE, NORMAN A. 1937. *Sorex dispar* in New Hampshire. Jour. Mamm. **18:** 95.
RICHMOND, NEIL D., AND WILLIAM C. GRIMM. 1950. Ecology and distribution of the shrew *Sorex dispar* in Pennsylvania. Ecology **31:** 279–282.
RIDGWAY, ROBERT. 1912. Color Standards and Color Nomenclature. Pub. by the author: Washington, D. C. iii + 43 pp., 53 plates.
TATE, G. H. H. 1935. Observations on the big-tailed shrew (*Sorex dispar* Batchelder). Jour. Mamm. **16:** 213–215.
STARRETT, ANDREW. 1954. Longtail shrew, *Sorex dispar*, in Maine. Jour. Mamm. **35:** 583–584.
WILSON, L. W., AND J. E. FRIEDEL. 1942. A list of mammals collected in West Virginia. Proc. West Va. Acad. Sci. **15:** 85–92.

NESTS AND YOUNG OF THE SIX-LINED RACERUNNER *CNEMIDOPHORUS SEXLINEATUS* LINNAEUS

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INTRODUCTION.—Present literature contains many brief references to the habits and habitats of the widely-ranging six-lined racerunner, *Cnemidophorus sexlineatus*. However, very few facets of the natural history of this lizard can be said to have received extensive coverage.

Barden (1942) observed the diurnality of the activity cycle and experimented with the cycle under various laboratory conditions. Taylor (1935) noted the time of activity in mid-summer and described burrows. The latter were also noted by Blanchard (1922). Cook (1943) stated that eggs were sometimes laid in what appeared to be specially prepared offshoots from mole runs. Pearson and Nelson (1952) called attention to the frequent use by this lizard of the burrows of *Peromyscus polionotus* in Florida, with the digging of side tunnels from the mouse burrows. In the Okefinokee region Wright and Funkhouser (1915) noted burrows in ploughed fields and stated that 4 or 5 eggs (about 16 x 10 mm.) were laid in holes 4 to 12 inches deep. Brimley (1939–1943) described the eggs of North Carolina specimens as larger, smoother and whiter than those of the fence lizard; 17–22 x 11–14 mm. in size; and laid in the ground in June and July in lots of 3 or 4. Definite egg sets have been recorded from widely-scattered points in the range of the species by Force (1930: 1 set, 4 eggs), Boyer and Heinze (1934: 3 sets, 4, 5 and 6 eggs), Hudson (1942: 1 set, 4 eggs), Cook (1943: 2 sets of 6 eggs [one of which, because of embryo size, would appear to have been 2 sets of 3 eggs]), Minton (1944: 6 eggs in a female), and McCauley (1945: 1 set, 3 eggs). Noble and Bradley (1933) described mating behavior as induced in laboratory specimens from Mississippi and Georgia. Food habits have been investigated by Force (1925: 18 stomachs), Burt (1928: 15 stomachs) and McCauley (1945: 10 stomachs). Hoyt (1941) made one observation on the speed of a specimen. Dillon and Baldauf (1945) and Stille (1947) recorded instances in which

disturbed specimens sought cover in nearby bodies of water. Carr (1940) and Neill (1948) noted hibernation sites. Available information on the habits of the species has been summarized by Burt (1928 and 1931), Pope (1944) and Smith (1946 and 1950).

METHODS.—During the summers of 1945, 1949, 1950 and 1952 the writer made studies of nests, eggs and young, in a single type of habitat, in adjacent portions of Mecklenburg, Iredell and Cabarrus Counties in piedmont North Carolina. The six-lined racerunner is one of the most abundant and characteristic lizards of this region, being partial to open fields, thicket margins and broken wooded areas. Farmers reported that they occasionally plowed up eggs (probably of this species), especially in the more sandy portions of fields. However, a type of habitat was sought that might provide a reasonable concentration of nests. The sawdust piles (mostly pine) of small and medium size, so characteristic of the southeastern states, were found to provide such an habitat. Although the sawdust piles were used to a limited degree by *Eumeces fasciatus* and *Sceloporus undulatus hyacinthinus* for egg deposition, they seemed to provide a favorite nesting site for *Cnemidophorus*.

A "Blanchard snake rake" was found to be a light and convenient tool for digging into the sawdust piles. A swath, 12 to 18 inches deep vertically, was first cut across one side of the base of a pile. The worker would then dig carefully back and forth across this cut, a bite not more than one to two inches deep (horizontally) being taken with each vertical stroke. The pile was thus gradually "shaved" into, with the face of the cut slowly progressing into, and up, its side. Provided the cut was kept 12 to 18 inches deep, it was found that no egg sets would be missed. Working up to the peak of the pile in this gradual manner, very few eggs were damaged. A nest usually would be nicked slightly, and the bright white eggs would be sufficiently conspicuous to give ample warning before another stroke did further damage. Eggs were then carefully stowed away in moist sawdust in the shade, and nest data, temperature, time, etc., were recorded. The digging process was then continued. When the top of the pile had thus been reached by a cut up one side, a similar cut would be started up another face.

In the laboratory, egg sets were measured and, in some cases, weighed. It was found that eggs could be "candled" at the edge of a shielded light and that reasonably satisfactory observations could sometimes be made as to the approximate degree of development of the embryo (especially during early stages). Any such manipulations had to be performed with speed and care. A few minutes' drying might cause an egg to burst, and either drying or rough handling might cause far advanced eggs to hatch prematurely. In one or two instances the mere disturbance of uncovering the nest site in a sawdust pile caused eggs which were on the point of hatching to do so immediately (within a few seconds).

Egg sets were buried in moist sawdust in stacked finger bowls or other containers. Several types of small cheap plastic food storage containers, obtainable from the ten-cent store and having closely-fitting lids, were found to be entirely satisfactory for this purpose. The full containers were placed on a table in a warm upstairs laboratory with open windows. Temperatures recorded at the

containers at various times of day and night during the incubation period varied from 82° to 93°F. and apparently averaged about 86°.

LOCATION OF NESTS.—The writer secured 64 sets of eggs from 8 sawdust piles in the course of several years. The largest number of sets secured from a single pile during one season was 12. Several of the piles investigated yielded no eggs at all. The reasons for the preference of the racerunner for certain piles are unknown, but several factors might be suggested: (1) The general location of the pile. (2) The alleged gregariousness of the racerunner. (3) Temperature relationships within the pile. The writer believes that some piles have internal temperatures sufficiently high to discourage egg deposition. (4) The age of the pile and its degree of decomposition and invasion by fungi. In the writer's experience a pile does not remain productive longer than four or five years. (5) Changes in the surrounding habitat. When the pile is first formed its environs may be relatively open. However, as thick vegetation grows up around the pile, the habitat becomes less suitable for the racerunner.

Most of the racerunner nests found were placed in the south, east or west sides of the sawdust piles. This was probably due simply to standard sawmill practices. The north, or north-east, side is likely to be obstructed by slabs and wood fragments. The more unobstructed portions of the piles seemed to be preferred by the lizards. In no instance was a nest found under a slab or piece of wood. This is worthy of note because one or two of the ground-placed nests reported in the literature were so located.

The sawdust forming the surface layers of a pile was usually dry to a depth of one or two inches. All sawdust below such a level was usually moist, regardless of the prevailing weather conditions, except in occasional areas where "dry rot" had set in. In only two instances were eggs found in dry areas of a pile. In three other cases the nest cavities were just within the moist zone, being more or less roofed by dry sawdust. In all other cases the nests were well within the moist portion of the pile. Whether the two "dry-placed" egg sets noted above would have hatched naturally is not known. One of these sets exhibited a considerable degree of egg shrinkage. In the laboratory both sets were placed in moist sawdust. Both hatched, 32 and 47 days later, respectively.

The depth of a nest cavity in the pile was measured from the floor of the cavity (position of the eggs) to the nearest point on the surface of the pile. In a series of 55 nests the depth of the nest cavity varied from 3 to 11 inches, with a mean of 5.6 inches and a standard deviation of 2.004. Only four of these nests were deeper than 8 inches. Thus, 51 (93%) were between 3 and 8 inches (inclusive), 38 (69%) were between 3 and 6 inches, and 28 (51%) were between 4 and 6 inches from the surface.

The racerunner is an expert and frequent burrower wherever a suitable medium presents itself. The writer has seen burrows used by this lizard in a pile of agricultural lime at the edge of a cultivated field, and one farmer near Davidson informed the writer that he had dug a set of lizard eggs out of such a pile of crushed limestone. The lizard digs into sawdust piles with great ease. No evidence has been observed that the same burrow may be used both for egg deposition and for

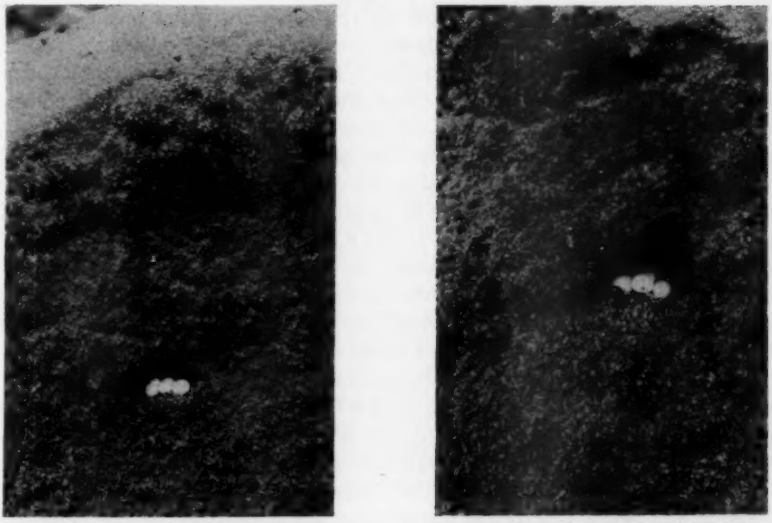


FIG. 1. Photographs of racerunner eggs as uncovered in the excavated face of a sawdust pile. The surface of the pile may be seen above in each case. The eggs of the farther set (left) are 6 inches deep; those of the nearer set (right) are 4.5 inches deep.

daily retreat. One even gains the impression that a burrow in sawdust may seldom be used more than once. Within a few seconds, a racerunner can thrust its snout through the surface of a pile and force its way out of sight. When this lizard digs with its limbs (as it does at times), it uses a one-arm method. Balancing itself on both hind limbs and one forelimb, it takes several successive strokes with the other forelimb. It then shifts its weight and takes several strokes with the first forelimb. This alternation is repeated.

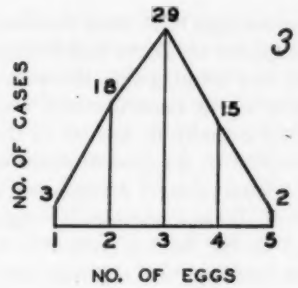
SIZE OF THE NEST CAVITY.—Egg sets were usually found in quite definite nest cavities, although occasionally such a cavity was not evident. A nest cavity is a low, closed, slightly dome-ceilinged chamber having perhaps twice (occasionally three times) the volume of its contained egg group. Chamber size varied somewhat with the number of eggs in the set. A fairly typical chamber for a set of 3 eggs would measure about 2 by 1¾ inches, with a ceiling height of 1 inch at the highest point. Eggs were placed loosely in this chamber, usually lying on their sides and very seldom in contact with the ceiling. Only very rarely were any of the eggs adherent to one another. In no case was the adult lizard found with the eggs. Nor was there usually any vestige of the tunnel by which the female had reached the surface of the pile after laying the eggs. The egg chamber was simply a cavity without evident entrance or exit remaining.

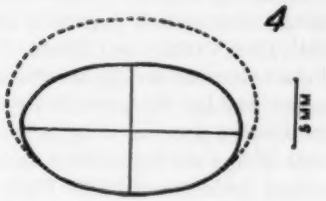
TEMPERATURE AT THE NEST.—The nests involved in this study were found at various times during daylight hours—some fairly early in the morning, many at about mid-day and many others during quite late afternoon. Temperatures of the sawdust at the egg level were carefully and quickly taken. These tempera-

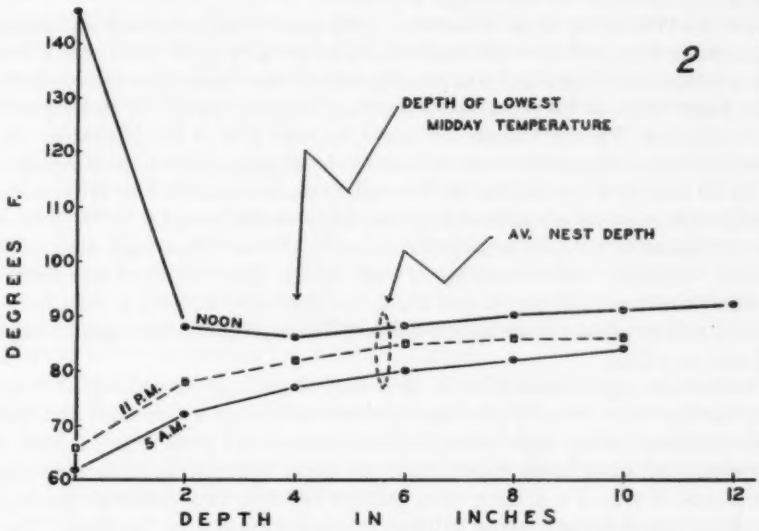
tures (as available for 55 nests) ranged from 84° to 96°F., with a mean of 90.8°F. The temperatures for 44 (80%) of these were between 88° and 94°F.

SIGNIFICANCE OF NEST DEPTH AND NEST TEMPERATURE.—The writer has not conducted an exhaustive investigation of sawdust pile temperatures in general. However, the data at hand seem to suggest certain definite features: (1) The *surface* of a sawdust pile is subject to very great extremes of temperature. On a hot summer day the surface temperature taken in the sun may be extremely high (i.e. 145°F.). Figure 2 records series of temperatures taken at various depths in the Egg Rock pile at noon and at 11:00 p.m. on July 5 and at 5:00 a.m. on July 6, 1952. These series are believed to represent reasonably typical mid-summer conditions. It will be noted that, between noon of one day and 5:00 a.m. of the next, the surface temperature of the pile showed a fluctuation of 84°F. (Air temperature 3 feet above ground varied only 23°F. during the same period.) (2) Within the interior of the sawdust pile the midday temperature drops abruptly. At a depth of 2 inches it may be down to between 88° and 100°F. (3) As the pile is penetrated more deeply, the temperature at first continues to fall until, at a depth somewhere between 4 and 6 inches, a level of minimal temperature in the midday temperature series will be reached. This low point will be perhaps 86°–90°F. (4) Below this *level of minimal midday temperature*, as one penetrates farther into the pile, the temperature again *rises* toward 100°F., (which temperature was reached in the present series at a depth of about 24 inches). (5) In the sawdust piles investigated the *average depth of nests* (5.6 inches) corresponded closely to the depth at which *minimal midday temperatures* were observed. If this is a coincidence, it is a very happy one. Happy because it places the eggs at a level subject to limited fluctuations in temperature, at a level of constant sawdust moisture, and at a level where the eggs are least likely to be subjected to temperatures of lethal intensity (although the exact lethal temperature level for incubating eggs is not known at present). (6) While the temperature at the surface of a sawdust pile might be subjected to a daily fluctuation of 80°F., the temperature at the level of average nest depth might experience a fluctuation of only about 8°F. (Fig. 2.)

The mechanism whereby the lizards seek out the fortunate egg-deposition level noted above is not known. It is possible that: (a) the lizard may simply seek to deposit within the zone of moist sawdust. Or (b) it may seek the area somewhat below the zone which is subject to marked daily fluctuations in temperature. This assumption requires the further assumption that the lizard remains at the site of deposition long enough to determine the zone of marked fluctuations. Or (c) as the racerunner is a highly diurnal species and as reptiles are highly sensitive to both moisture and temperature, it is possible that the lizard simply deposits during the day and seeks the mildest, or most comfortable, temperature level within the moist zone. (The actual time of deposition in nature is not known.) All the writer's records indicate that the characteristically high "midday" temperatures within the pile actually prevail by 9 to 10 o'clock on a summer morning and probably continue for at least ten hours thereafter. Such temperature conditions would certainly prevail on many a day in May or June when eggs might be laid.







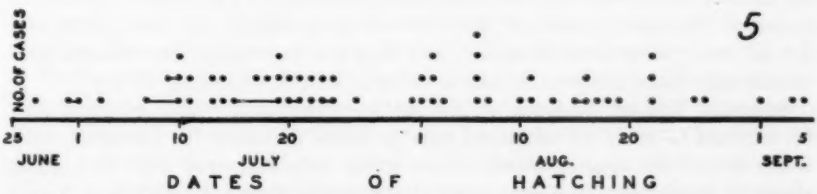


FIG. 2. Graph indicating the temperature at various depths in the Egg Rock sawdust pile at noon, 11:00 p.m., and 5:00 a.m. on July 5 and 6, 1952. Note the great variation at the surface of the pile, the relatively slight variation at depths below 4 inches. FIG. 3. Graph indicating the number of eggs found in 67 sets. FIG. 4. Increase in dimensions of an average egg during incubation. Based upon measurements of 16.2 x 9.3 mm. at laying, and 18.4 x 13.0 mm. at hatching. FIG. 5. Graph indicating the dates of hatching of 55 sets of eggs in the laboratory. Two dots joined by a line indicate that two or more days were required for the completion of hatching.

NUMBER OF EGGS IN A SET.—Sets of as many as six eggs have been recorded from the more central portion of the range of this species by Boyer and Heinze (1934), Cook (1943), and Minton (1944). It seems very striking that the writer did not encounter an egg set this large in the region of the present study. One wonders whether the productivity of this form may actually be greater in the more western portions of its range. Figure 3 shows that in the present series of 67 sets of eggs the egg number varied from 1 to 5, with a mean of 2.9 eggs and a standard deviation of 0.885. Forty-three per cent of the sets contained 3 eggs, and 92.5 per cent contained either 2, 3 or 4 eggs. So far, having been able to observe no evidence to the contrary, the writer has assumed that a female racerunner deposits only one set of eggs per season.

SIZE AND WEIGHT OF EGGS AT LAYING.—The eggs of the racerunner are usually ellipsoidal in form, soft and thin-shelled and, as Brimley (1939–1943) has noted, they are somewhat larger and considerably whiter than those of *Sceloporus undulatus*. Eggs taken in the field had undergone varying degrees of development when collected. Therefore those few eggs that were laid in the laboratory presented the most dependable data concerning dimensions at the time of laying.

The 10 eggs of 3 sets laid in the laboratory varied from 15.1 to 17.0 mm. in length, with an average length of 16.2 mm. They varied from 8.2 to 10.0 mm. in greatest diameter, with an average diameter of 9.3 mm. These eggs were examined by "candling" within one day of their laying. They appeared to exhibit a minute blastoderm between 4 and 5 mm. in diameter, without a definite embryonal area or blood vessels as yet evident. These 10 laboratory eggs averaged 0.78 gm. in weight.

The freshest eggs collected in the field were 6 specimens belonging to 3 sets. They hatched from 54 to 57 days later and were evidently within their first week of development. They appeared to exhibit blastoderms from 5 to 6.5 mm. in diameter, with small blood vessels visible in some, and with an embryonal area reminiscent of that of a 33-hour chick embryo visible in two. Although the heart may have been beating in some of these, it was not evident in "candling." (In 2 additional specimens which hatched 52 days later, the blastoderm measured approximately 7 by 9 mm. and the heart beat was evident.) The 6 eggs noted above averaged 17.3 mm. (variation: 16.5–18.0 mm.) in length, 9.2 mm. (variation: 9.0–9.5 mm.) in greatest diameter, and 0.84 gm. in weight. (One month later these 6 eggs had slightly more than doubled in weight, averaging 1.8 gm.)

SIZE AND WEIGHT OF EGGS AT HATCHING.—Surprisingly little handling may be required to cause far-advanced eggs to hatch prematurely. Therefore, relatively few of the eggs involved in this study were tampered with during late stages of incubation. Ten eggs from 4 sets, which were measured from 1 to 4 days before hatching, exhibited an average length of 18.4 mm. (range: 17.–21. mm.) and an average diameter of 13. mm. (range: 11.7–13.5 mm.). Figure 4 indicates graphically the increase in egg dimensions during incubation. Comprehensive figures on weights of eggs just prior to hatching were not obtained. Four eggs of two sets weighed at this time averaged 1.76 gm. The writer did not record the complete and continuous story of egg weights throughout the period of incubation.

SEASON OF HATCHING.—Figure 5 records the dates of hatching of 55 sets of eggs that were incubated in the laboratory during the four summers of the study. In cases in which broods of young required more than one day to complete the hatching process, horizontal lines are used instead of dots to indicate this fact. It will be seen that the earliest date of emergence was June 27 and the latest was September 5. However, only 5 broods hatched before July 10 and only 5 hatched after August 22. Of the 55 broods, 45 (82%) hatched between July 10 and August 22, and 36 (65%) hatched between July 10 and August 11.

PROBABLE LENGTH OF THE INCUBATION PERIOD.—It appears likely that the incubation period for eggs of the racerunner may have a duration of 8 to 9 weeks. The longest recorded incubation period—that for a set of eggs laid in the laboratory—was 61 days. Three other sets of eggs, having blastoderms of from 5 to 6.5 mm. in diameter when found, required from 54 to 57 days to complete development. Various other sets of eggs, whose development was obviously more advanced at the time of collection, required proportionately shorter periods of time to reach the hatching point. It has been pointed out that the incubation of eggs in the laboratory was carried on at temperatures varying from 82° to 93°F. and averaging perhaps 86°F. Also, it is evident that eggs incubated in natural nests in sawdust piles must have undergone temperature fluctuations varying at least from 80° (or below) to 96°F. (average unknown). It seems evident that some variation in the length of incubation *in nature* should be expected. It would be relatively easy to incubate eggs at a constant temperature in the laboratory. However, it is questionable whether the results would be comparable to those achieved under fluctuating conditions in nature.

PROBABLE SEASON OF EGG-LAYING.—With an incubation period of about 60 days and a major period of hatching during July and early August, it appears evident that the majority of egg sets are deposited during May and early June. It appears likely that lower average temperatures during May may lengthen the period of incubation for the earlier sets.

SEX RATIO AT HATCHING.—It was found that, by means of a combination of pressure at the base of the tail, probing and observation of the general conformation of the vent, the sex of young racerunners could be determined with a fair degree of ease. Out of 108 young from 38 broods there were 56 males and 52 females. This would give a ratio of 108:100 for this limited series of young.

MORTALITY OF YOUNG DURING LABORATORY INCUBATION.—Of 151 eggs incubated in the laboratory, 20 failed to hatch and 1 young lizard died immediately after hatching. The degree of mortality during incubation was thus 21 individuals out of a potential 151, or 14 per cent. The 20 hatching failures were presumed to be due to infertility and other causes unknown. Only one individual from the potential 151 hatched in a noticeably deformed condition. This specimen had an umbilical scar extending most of the length of the belly (usual length = 2 scale rows), its back was somewhat arched, and the pelvic limbs were deformed in such a manner that walking was accomplished on the heels and inadequate purchase was secured against the substratum. It seems likely that this individual would not have survived.

SIZE AT HATCHING.—Measurements for 28 newly-hatched male lizards from

19 broods and 28 females from 18 broods may be averaged as follows, with the range for each measurement also indicated. These measurements show that there is no appreciable difference between the sexes. Measurements are in millimeters:

	Males	*Females*
Total length	93.7 (87.–100.)	93.2 (85.–101.)
Snout-vent	32.3 (31.–34.)	32.6 (31.–34.5)
Tail length	61. (56.–66.)	60.5 (53.–66.5)
Head length	9.6 (9.–10.)	9.6 (9.5–10.)
Head width	5.9 (5.5–6.5)	5.9 (5.5–6.3)

WEIGHT AT HATCHING.—Thirty-one newly-hatched male racerunners from 18 broods showed an average weight of .845 gm. Twenty-nine newly-hatched females from 19 broods had an average weight of .842 gm. With the above average weight (.84 gm.) of an hatchling lizard in mind, and assuming the approximate correctness of the previously noted weight of an egg (1.76 gm.) just prior to hatching, it was calculated that the body of the young lizard would comprise approximately 48 per cent of the weight of an egg at the time of hatching, while the remaining 52 per cent of egg weight would be devoted to extra-embryonic membranes, extra-embryonic liquid and shell.

PRODUCTIVITY OF A BREEDING FEMALE.—It is desirable to have available an estimate of average productivity of a racerunner female, for purposes of comparison with findings in other reptilian species. The average female, immediately after laying, weighs about 8.5 grams. She has produced 3 eggs, weighing 2.34 grams, thus about 27 per cent of her weight. The young hatching from these eggs weigh 2.52 grams, almost 30 per cent of her weight.

SUMMARY

(1) Nests of the six-lined racerunner, *Cnemidophorus sexlineatus*, were found in considerable number in sawdust piles in the vicinity of Davidson, North Carolina. (2) The average nest cavity measured 2 x 1¾ inches, with a depth of 1 inch. It was located 5.6 inches (extremes: 3 to 11 inches) beneath the surface of the pile, within the zone of moist sawdust, and beneath the level of marked fluctuations in daily temperature. Temperatures at the nest sites, recorded for 55 nests at the time of collection, averaged 90.8°F. (3) During one 17-hour period in mid-summer, a fluctuation of 80°F. was recorded at the *surface* of a sawdust pile; in the same period the temperature at the level of average nest depth showed a fluctuation of only about 8°F. (4) The average set of 2.9 eggs (extremes of 67 sets: 1 to 5 eggs) occupied the next cavity loosely and was not brooded by the female. (5) One hundred and fifty-one eggs carried through the incubation period in moist sawdust in the laboratory exhibited a mortality of 14 per cent. (6) The *average* ellipsoidal, whitish egg measured at laying 16.2 x 9.3 mm. and weighed 0.78 gm. Just before hatching this egg measured 18.4 x 13 mm. and weighed 1.76 gm. (7) Among 55 sets of eggs the earliest date of hatching was June 27, the latest September 5. Eighty-two per cent of these sets hatched between July 10 and August 22. (8) Available evidence suggests a post-laying in-

cubation period of approximately 60 days. Eggs were probably laid most extensively during May and early June. (9) The sex ratio in 108 young from 38 broods was 108:100. (10) The average newly-hatched young lizard had a total length of 93 mm., a snout-vent length of 32 mm., a tail length of 61 mm., a head length of 9.6 mm., a head width of 5.9 mm., and a weight of 0.84 gm., equivalent to approximately 48 per cent of the weight of the average egg just before hatching. (11) The average female, after laying, weighs about 8.5 gm. She has produced eggs having a weight equivalent to about 27 per cent of her weight, and from these eggs hatch young having a collective weight equal to about 30 per cent of her weight.

LITERATURE CITED

BARDEN, ALBERT. 1942. Activity of the lizard, *Cnemidophorus sexlineatus*. Ecology **23:** 336–344.

BLANCHARD, FRANK N. 1922. The amphibians and reptiles of western Tennessee. Occ. Pap. Mus. Zool. Univ. Michigan No. 117: 1–18.

BOYER, DOROTHY A., AND A. A. HEINZE. 1934. An annotated list of the amphibians and reptiles of Jefferson County, Missouri. Trans. Acad. Sci. St. Louis **28:** 185–200.

BRIMLEY, C. S. 1939–1943. Amphibians and reptiles of North Carolina. Carolina Tips, Installment No. 17. Elon College, N. C.: Carolina Biol. Supply Co.

BURT, CHARLES E. 1928. The lizards of Kansas. Trans. Acad. Sci. St. Louis **26:** 1–85.

———. 1928. Insect food of Kansas lizards with notes on feeding habits. Jour. Kansas Ent. Soc. **1:** 50–68.

———. 1931. A study of the Teiid lizards of the genus *Cnemidophorus* with special reference to their phylogenetic relationships. Bull. U. S. Nat. Mus. **154:** 1–286.

CARR, ARCHIE FAIRLY. 1940. A contribution to the herpetology of Florida. Univ. Florida Pub., Biol. Sci. Ser. **3:** 1–118.

COOK, FANNYE A. 1943. Alligators and lizards of Mississippi. Mississippi Game and Fish Comm., Survey Bull. Pp. v + 20 (mimeo).

DILLON, LAWRENCE S., AND RICHARD J. BALDAUF. 1945. Unusual behavior of *Cnemidophorus sexlineatus*. Copeia 1945(3): 174.

FORCE, EDITH R. 1925. Notes on reptiles and amphibians of Okmulgee County, Oklahoma. Copeia **141:** 25–27.

———. 1930. The amphibians and reptiles of Tulsa County, Oklahoma, and vicinity. Copeia 1930(2): 25–39.

HOYT, J. SOUTHGATE Y. 1941. High speed attained by *Cnemidophorus sexlineatus*. Copeia 1941(3): 180.

HUDSON, GEORGE E. 1942. The amphibians and reptiles of Nebraska. Univ. Nebraska Cons. and Surv. Div., Cons. Bull. **24:** 1–146.

MCCAULEY, ROBERT H., JR. 1945. The Reptiles of Maryland and the District of Columbia. Hagerstown, Md.: Pub. by the Author.

MINTON, SHERMAN, JR. 1944. Introduction to the study of the reptiles of Indiana. Amer. Midland Nat. **32:** 438–477.

NEILL, WILFRED T. 1948. Hibernation of amphibians and reptiles in Richmond County, Georgia. Herpetologica **4:** 107–114.

NOBLE, G. K., AND H. T. BRADLEY. 1933. The mating behavior of lizards; its bearing on the theory of sexual selection. Ann. New York Acad. Sci. **35:** 25–100.

———, AND H. K. TEALE. 1930. The courtship of some iguanid and teiid lizards. Copeia 1930(2): 54–56.

PEARSON, PAUL G. AND GID E. NELSON, JR. 1952. Some commensal relations of the lizard *Cnemidophorus sexlineatus* (Linne). Copeia 1952(3): 188–189.

SMITH, HOBART M. 1946. Handbook of Lizards. Ithaca, N. Y.: Comstock Pub. Co. Pp. xxi + 557.

———. 1950. Handbook of Amphibians and Reptiles of Kansas. Univ. Kansas Mus. Nat. Hist., Misc. Publ. No. 2: 1–337.

STILLE, W. T. 1947. The aquatic habits of *Cnemidophorus sexlineatus*. Copeia 1947(2): 143.

TAYLOR, EDWARD H. 1935. Arkansas amphibians and reptiles in the Kansas University Museum. Univ. Kansas Sci. Bull. **22**: 207–218.

WRIGHT, ALBERT H., AND W. D. FUNKHOUSER. 1951. A biological reconnaissance of the Okifinokee Swamp in Georgia. The reptiles. I. Turtles, lizards and alligators. Proc. Acad. Nat. Sci. Philadelphia **67**: 107–139.

NOTES ON AMPHIBIANS FROM THE UPPER COASTAL PLAIN OF NORTH CAROLINA

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The published lists of Brimley (1940) and Brandt (1936) indicate few amphibian records for Moore or Richmond counties, North Carolina. This area includes the southwestern part of the upper Coastal Plain of North Carolina, the Fall Line marking off approximately the northwestern third of Moore County and cutting through the extreme western part of Richmond County. The underlying geological formations are presumably of late Pliocene or early Pleistocene age and include the much dissected and poorly defined "Lafayette" terrace deposits of Hay (1923, Map 39) or the "High Level Gravels" of Richards (1950). These sediments consist of light grey to yellow, orange, or red, mixed sands, gravels, and clays. The relief is greater than that usually encountered on the lower terraces of the Coastal Plain.

In the vicinity of Southern Pines and Pinehurst, in Moore County, there are well developed pine barrens with longleaf pine (*Pinus palustris*) as the dominant form. Various oaks, including Spanish oak (*Quercus falcata*) and black jack oak (*Quercus marilandica*) are important in the understory. The swamps of the area are dominated by water tupelo (*Nyssa aquatica*) and sweet gum (*Liquidambar styraciflua*). In addition to the pine barrens and swamps there are extensive areas of mixed deciduous growth, cultivated fields, and other habitats typical of places in agricultural use or that recently have been disturbed.

The region between Rockingham and Everett's Pond, just north of the South Carolina border, consists in part of well drained agricultural land and in part of deciduous or mixed deciduous-coniferous woodland and scrub.

The principal breeding habitats for tailless amphibians in the barrens are small grassy pools and sand pits, the backwaters of small streams, and fair-sized ponds partly bordered by swamps, and at least partly artificial. Outside the pine lands are breeding places in partly flooded pastures, farm pools and ponds, and deciduous swamps.

In early July, 1954, we visited this area for the purpose of collecting amphibians and especially seeking *Hyla andersoni*. Our time was spent within a radius of 15 miles north and west of Southern Pines in the southern part of Moore County. In addition, we collected between Rockingham and the South Carolina border along U.S. Route 1 in southern Richmond County. On the afternoon of our arrival at Southern Pines there was a very heavy thunder shower in the area and there was rain during the following evening. There was also a violent shower on July 5 at Rockingham. According to residents these were the first rains in several weeks. 45 frogs, 3 salamanders, and several lots of tadpoles were collected, representing 10 species.

In spite of the short time spent in the area and the small amount of material collected, the data obtained are of interest in connection with the distribution of the amphibians of the Coastal Plain, and with regard to the problems involving *Bufo terrestris* and *Bufo fowleri*. Except for the two *Hyla andersoni* series (AMNH *A22418–21*, *A35150–55*) which were borrowed from the American Museum of Natural History, the materials discussed are preserved in the Newark Museum. Thanks are due to Mr. C. M. Bogert of the American Museum for reading this manuscript and for the loan of the materials mentioned.

Scaphiopus holbrooki holbrooki.—An adult female, 51.5 mm. in snout-vent length, with the dorsal pattern completely obscured, was taken on Route 1 between Rockingham and Everett's Pond on July 5 in the early evening. The oviducts were not enlarged and the ovaries were without ripe eggs.

Bufo.—23 toads were obtained including 10 adults from the Southern Pines region and one adult from Everett's Pond, the rest being juveniles.

Burt (1938) and Brimley (1940) reviewed the records for North Carolina toads. The *Bufo terrestris americanus* records are all within the Blue Ridge physiographic province except for records from Burke County, immediately adjacent to this province, and from Wayne County which straddles the Fall Line. Records for *B. t. terrestris* are confined to the Coastal Plain and are principally from the lower terraces. The records (except for the Wayne County record for *americanus*) indicate a disjunct distribution of the two forms in North Carolina. The distribution of *Bufo woodhousi fowleri* is given as being statewide although there are no records from the extreme southeastern counties.

Blair (1941) indicated hybridization between *terrestris* and *fowleri* in a York (Sumter County), Alabama population, and Blair (1947) says that intermediates are found along the line of junction of the two forms elsewhere in the southeast. Other authors mention similar tendencies but usually fail to give specific details of such populations. For this reason a discussion of the Moore County material may be of some interest.

B. t. terrestris and *B. w. fowleri* were both heard in chorus in Moore County; at Thaggard Pond, north of Southern Pines, and at a roadside pond between Southern Pines and Carthage. The sample of 10 adults discussed contains specimens from the two localities. Mainly on the basis of the presence or absence of conspicuous knobs on the supraorbital crests (supposedly diagnostic of *terrestris*), these specimens were divided into two equal groups. The *fowleri*-like series was designated Group A, and the *terrestris*-like series was designated Group B. Several

of the Group A specimens were giving more or less typical *fowleri* calls when collected. For comparison we examined 15 adult *terrestris* from the vicinity of Folkston, Charlton County, Georgia, and 22 adult *fowleri* from southern New Jersey (6 different counties from Ocean County, south).

A number of additional characters were examined. Typical *fowleri* and Group A specimens lack enlarged warts on the thigh. Four of the five specimens in Group B had such warts, but the Folkston series of typical *terrestris* is highly variable in this character. Typical *fowleri* are usually less spinous than *terrestris*; the Moore County groups are variable in this respect. Individuals in both typical series of *fowleri* and *terrestris* vary in the character of spotting on the upper chest, some specimens being spotless, and others being quite coarsely spotted. The Moore County groups were equally variable. The variation in the development of the vertebral stripe seems equally random although the stripe is usually more sharply defined in *fowleri*.

Measurements were made as follows: length of preparotoid crest; maximum length and width of the parotoid; minimum interparotoid distance; and maximum and minimum separation of the supraorbital crests. Since the mean snout-vent lengths were similar in the two typical series (51 mm. for *fowleri*, 48 mm. for *terrestris*) the means of the other measurements of these series were compared directly. The interparotoid distances were nearly equal in both groups. However, the parotoids average somewhat larger (longer and wider) in *fowleri*. The preparotoid crest is well developed in most *terrestris* and is almost as useful as the supraorbital knobs in recognizing individual specimens. The anterior border of the parotoids in *fowleri* touches the parietal crest and the separation of the two is rather difficult to measure accurately, the preparotoid crest being poorly developed or absent. The supraorbital crests of *terrestris* are more widely spaced and are rather consistently divergent. These crests in *fowleri* are more nearly parallel and lack the conspicuous knob posteriorly.

Some of the above differences were tested statistically by using the following ratios: maximum separation of supraorbital crest/minimum; parotoid length/parotoid width; interparotoid distance/parotoid length; preparotoid crest or gap/minimum separation of supraorbital crests. Table I summarizes the data for the ratios. In each case significant differences were found between typical *terrestris* and *fowleri* ratios. Values of $d/S.E._d$ greater than 3 were taken to indicate a significant difference between two means (Mayr, *et al.* 1953). No significant differences were found between Group A (*fowleri*-like) and typical *fowleri*. Group A differs significantly from typical *terrestris* in the ratios: interparotoid distance/parotoid length, and preparotoid gap/minimum separation of supraorbitals. One Group A specimen, while *fowleri*-like in other respects, including call, has *terrestris*-like supraorbital knobs. Group A agrees fairly well in the characters examined with typical *fowleri*.

The agreement of the Group B (*terrestris*-like) series with typical *terrestris* is less satisfactory. This sample does not differ significantly in the ratios examined from either Group A or typical *fowleri*. The supraorbital knobs are about as well developed as in typical *terrestris* but Group B differs significantly from the typical

TABLE I

Variation in four characters in Bufo w. fowleri, B. t. terrestris, *and 2 lots of specimens from Moore County, N. C.*

	n	m	range	S.D.
Parotoid length/width				
fowleri	22	2.26	1.46–3.24	.31
group A	5	2.16	1.97–2.45	.22
group B	5	2.03	1.72–2.25	.22
terrestris	15	2.00	1.73–2.37	.18
Supraorbital crests. maximum separation/minimum				
fowleri	22	1.45	1.17–1.71	.13
group A	5	1.46	1.25–1.67	.17
group B	5	1.54	1.39–1.59	.15
terrestris	15	1.85	1.45–2.24	.29
Interparotoid space/parotoid length				
fowleri	22	.85	.75–.99	.23
group A	5	.90	.81–1.02	.09
group B	5	.90	.70–1.13	.16
terrestris	15	1.04	.87–1.26	.10
Preparotoid crest/minimum separation of supraorbital crests				
fowleri	22	.14	.04–.31	.06
group A	5	.25	.06–.53	.18
group B	5	.34	.17–.47	.16
terrestris	15	.60	.32–.76	.11

form in regard to the ratio, preparotoid crest/minimum separation of supraorbitals.

In summary, the Group B sample seems to represent an example of *terrestris* x *fowleri* interbreeding. The characters examined vary more or less independently and individual specimens may have mixtures of characters of both end forms. The Moore County samples are small and the need for more extensive study is indicated. On the basis of summaries of North Carolina *Bufo* records in the literature we have eliminated *B. t. americanus* from the present discussion. It is possible that the toads of the Fall Line area in North Carolina are also influenced by gene flow from this third source. Mention of *B. t. terrestris* elsewhere in this paper refers to the type of call heard.

The habitats from which *fowleri* calls were heard include lake shores and small, swamp-bordered farm ponds. None of these situations was in "good" barrens. *B. t. terrestris* called from the same situations, and also from small ponds and sand pits in the barrens.

Acris gryllus gryllus.—11 specimens from the vicinity of Southern Pines were assigned to this form. *Acris* was calling from a great variety of habitats both within and outside the barrens.

Hyla andersoni. 2 adult males, 33.1 mm. and 35.8 mm. in snout-vent length, were collected between Niagara and Thaggard's Pond, north of Southern Pines, Moore County, on July 4, 1954. The single small chorus of *H. andersoni* that we were able to locate was calling from a hillside covered with scrub growth and a few scattered trees. The slope was quite steep and had an east-southeast exposure. According to a local resident the situation is "always wet". The principal ground cover was a well saturated sphagnum mat lying on a sandy clayey soil. *Acris* was the only other anuran present. The herbaceous growth included pipewort (*Eriocaulon*), orange milkwort (*Polygala lutea*), sweet pepperbush (*Clethra sp.*), and sundew (*Drosera sp.*). The shrubs included azalea (*Rhododendron sp.*), blueberry (*Vaccinium sp.*), and alder (*Alnus sp.*). Among the trees were longleaf pine (*Pinus palustris*), blackjack oak (*Quercus marilandica*), and red maple (*Acer rubrum*). The specimens taken were calling from blueberry bushes 2 or 3 feet above the ground. No other choruses of *Hyla andersoni* were located either in the Southern Pines-Pinehurst area or at Everett's Pond where Wright and Wright (1949) found this species.

The geological relationships of localities in which *H. andersoni* populations have been found may be of some interest. In New Jersey the principal pine barrens area lies on formations (Cohansey, Kirkwood, and Beacon Hill remnants) of Miocene to Late Pliocene Age (Kummel, 1940). The North Carolina populations are on "Lafayette" or "High Level Gravel" formations of Pliocene or early Pleistocene Age (Richards, 1950). Neill (1948) discovered a population in Richmond County, Georgia. This county lies at the edge of the Fall Line and the underlying formations are presumably (Schuchert, 1943, Fig. 54) of Upper Cretaceous or early Tertiary Age. The occurrence of *H. andersoni* on the older part of the Coastal Plain and its presumed absence on the younger part of the Coastal Plain may have significance in considering the history of this apparently relict form.

In order to determine whether the disjunct distribution of *H. andersoni* involves pronounced morphological variation, we made comparisons between a series of 13 specimens from the Southern Pines area (11 American Museum of Natural History specimens plus our own 2) and 26 specimens collected in New Jersey (representing 8 central and southern counties). No distinct differences were observed in markings or coloration in the preserved material. Measurements were made of body length from snout to vent; head length from tip of snout to rear of the tympanum; head width immediately behind the tympana; tibia length with the knee flexed; total leg length from vent to tip of the longest toe with the leg extended, and greatest diameter of the tympanum. The tympanum varies considerably in size in both samples and a statistical comparison of the two populations with regard to this character was not made. Ratios derived from the above measurements were as follows: body length/head length, head width/head length, body length/tibia, total leg length/tibia, total leg length/body length. A statistical comparison was made of the 10 males in the North Carolina series and the 20 males in the New Jersey series. Since the body length means in the two samples were very close (35.8 mm. for New Jersey and 35.1 mm. for

North Carolina) a rough comparison was made of the actual measurements. These indicate a greater length of head, tibia, and leg in the southern material. Although definitely significant differences ($d/S.E._d$ equal to 4 or more) were obtained for the means of two ratios (Fig. 1) we do not see any useful purpose in distinguishing the two populations with a nomenclatural change.

Hyla cinerea cinerea. No specimens were collected but this form was heard in strong chorus at Everett's Pond and at Rockingham. This species was not heard in the Southern Pines area.

Hyla femoralis. An adult male and a male and female in amplexus were taken at the edge of a shallow, sandy pool in well developed pine barrens a short distance west of Southern Pines. Males were calling from the ground and from low bushes. The female contained ripe eggs. Associated species were *Acris g. gryllus*, *Bufo t. terrestris*, and *Triturus viridescens*. The pool contained very little vegeta-

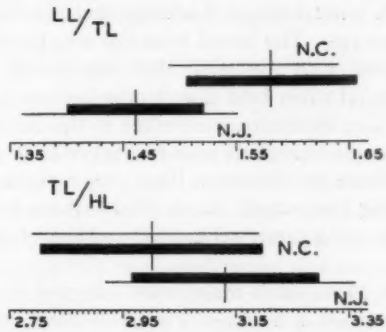

FIG. 1. Variation in the ratios: leg length/head-body length (above) and head-body length/head length (below) in two populations of *Hyla andersoni*. 10 males from Moore County, N. C. are compared with 20 males from New Jersey. The thin horizontal lines indicate the observed range; the vertical line, the mean; and the heavy horizontal line, one and one-half times the standard deviation.

tion but was well stocked with *Odonata* larvae, diving beetles (*Dytiscidae*) and backswimmers (*Notonectidae*).

A single lot of *H. femoralis* larvae, varying in size up to about 25 mm. in length, was also taken from this pool. The tail fins of many of these tadpoles, and especially of the larger specimens, were strongly colored with red-orange and with heavy dark blotches near the upper edge, similar to the coloration of mature larvae of *Hyla versicolor*. The mature *H. femoralis* larvae contrast with those of the latter species in having a sharply striped tail musculature. *H. femoralis* has formerly been listed only from the lower Coastal Plain in North Carolina, (Brimley, 1940) and this population extends the range of the form inland almost to the Fall Line.

Hyla squirella. One adult male collected in a large, partly flooded pasture on Little Creek Farm between Southern Pines and Carthage. The mixed chorus at

this locality consisted of *Bufo t. terrestris*, *Microhyla carolinensis carolinensis* and *Hyla squirella* in order of vocal intensity. The *H. squirella* taken was on the ground among matted rushes. This species is recorded as abundant at Columbia, South Carolina on the Fall Line (Corrington, 1929) but previous North Carolina records are mainly from the lower Coastal Plain. (Brimley, 1940).

Rana catesbeiana. No specimens of this species were taken but it was heard with *Acris*, *B. w. fowleri*, *B. t. terrestris*, and *Rana clamitans* at a small roadside pond between Carthage and Southern Pines. The locality was in an area of cultivated fields and mixed deciduous growth.

Rana clamitans. An adult male, a small adult female (61 mm. snout–vent length), and a juvenile were collected on U. S. Route 1 between Rockingham and Everett's Pond on the evening of July 5. The female had the ovaries well packed with ripe eggs.

A group of mature tadpoles, identified as belonging to this species, were collected from a roadside pond between Carthage and Southern Pines (see notes under *R. catesbeiana* above). The larval behavior was peculiar. They hid close to shore when disturbed, with the result that they would readily seek shelter under the collector's hand when held close to the bottom of the pond. Most of the series of several dozen specimens were taken in this manner.

Microhyla c. carolinensis. One adult male was taken from a chorus in a flooded pasture between Carthage and Southern Pines (see notes under *Hyla squirella*). Individuals were calling from small, water-filled furrows in the field, and were very difficult to locate, being partly submerged and hidden under matted grasses and rushes.

Triturus viridescens. Three adult males were collected in a sandy pool in well developed pine barrens west of Southern Pines (see notes under *Hyla femoralis*). Two of the adults have a pattern of broken lateral stripes. The third is marked with large, closely spaced red spots. Bishop (1943) mentions Moore County as an intergrade area between *T. v. viridescens* and *T. v. dorsalis*.

LITERATURE CITED

BLAIR, A. P. 1941. Variation, isolating mechanisms, and hybridization in certain toads. Genetics **26:** 398–417.

BLAIR, A. P. 1947. Variation of two characters in *Bufo fowleri* and *Bufo americanus*. Amer. Mus. Novit. **1343:** 1–5.

BISHOP, S. C. 1943. Handbook of Salamanders. Comstock Publishing Company: Ithaca, New York.

BRANDT, B. B. 1936. The frogs and toads of eastern North Carolina. Copeia **4:** 215–223.

BRIMLEY, C. S. 1940. The amphibians and reptiles of North Carolina. Carolina Tips, **10:** unnumbered pages.

BURT, C. E. 1938. The frogs and toads of southeastern United States. Trans. Kansas Acad. Sci. **41:** 331–360.

CORRINGTON, J. D. 1929. Herpetology of the Columbia, South Carolina region. Copeia **172:** 58–83.

HAY, O. P. 1923. The Pleistocene of North America and its vertebrated animals from the states east of the Mississippi River and from the Canadian provinces east of longitude 95 degrees. Pub. Carnegie Inst. of Wash., No. 322. viii + 499 pp.

KUMMEL, H. B. 1940. The geology of New Jersey. Bull. N. J. Dept. Conserv. and Devel. (Geol. Ser.) No. 50. 203 pp.

MAYR, E., E. G. LINDSLEY AND R. L. USINGER. 1953. Methods and Principles of Systematic Zoology. McGraw Hill Book Company: New York.

NEILL, W. T. 1948. Hibernation of amphibians and reptiles in Richmond Co., Georgia. Herpetologica 4: 107–114.

RICHARDS, H. G. 1950. Geology of the coastal plain of North Carolina. Trans. Amer. Philos. Soc. (New Ser.) 40: 1–83.

SCHUCHERT, C. 1943. Stratigraphy of the Eastern and Central United States. John Wiley and Sons: New York.

WRIGHT, A. H. AND A. A. 1949. Handbook of Frogs and Toads of the United States and Canada. Comstock Publishing Company: Ithaca, New York.

THE SEPARATE EFFECTS OF FOOD AND TEMPERATURE DIFFERENCES ON DEVELOPMENT OF MARBLED SALAMANDER LARVAE[1]

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Larval populations of the marbled salamander (*Ambystoma opacum* Gravenhorst) in two temporary ponds in the vicinity of Chapel Hill, North Carolina, show very striking differences in growth and metamorphosis. The purpose of this study was to investigate possible differential effects of amount of food and degree of temperature on the length of the larval period and size at metamorphosis.

The problem was approached by observing the larval salamanders under controlled laboratory conditions until metamorphosis and by using these results to help explain concurrent observations made on animals collected from the two natural habitats.

The marbled salamander lays its eggs on land during the early fall in areas that will later be inundated. Eggs hatch when submerged; larvae then live through the winter months with little growth until spring when temperatures rise. After rapid increase in size, transformation into the terrestrial form occurs, usually in late May or early June, depending upon environmental conditions (Noble and Brady, 1933).

ACKNOWLEDGEMENTS.—I wish to express my appreciation to Dr. William L. Engels for suggesting the problem, for his guidance and encouragement during the course of the investigation, and for assisting in the preparation of the manuscript. Larval collections during the 1947–1948 season were made by Dr. Engels. I am indebted to Thomas G. Donnelly for advice on statistical analysis, and to Rinehart Baron for correction of the manuscript.

OBSERVATIONS UNDER CONTROLLED CONDITIONS.—One hundred *Ambystoma opacum* larvae were collected at random from Salamander Pool, near Chapel

[1] Condensed from a thesis submitted to the faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Zoology in 1951.

Hill, North Carolina, on January 7, 1950. The larvae, of similar developmental stages, had fore limbs with two to four toes and hind limb buds. Since larvae were observed in the pool on November 11, 1949, specimens could have been as much as two months old at the time of collection. Each larva was placed in spring water in a separate 300-ml. individually marked fingerbowl covered with a glass plate, and kept in a constant temperature room. Fifty larvae were put in a room averaging 20°C. and 50 in a room averaging 14°C. Both sets were further subdivided into groups of 25 larvae each; one was given abundant food, and the other was fed a minimal amount. The four groups were classified as follows: Group A: 14°C., well-fed; Group AA: 14°C., poorly-fed; Group B: 20°C., well-fed; and Group BB: 20°C., poorly-fed.

Larvae were fed copepods, ostracods, and amphipods the first month. After that, chopped tubifex worms were added to the diet until the larvae were large enough to eat whole worms. Abundant food was present at all times in the dishes of "well-fed" animals, allowing them to feed at will. "Poorly-fed" animals were given only what they could eat at the daily feeding. Within each group the amount of food given each larva was constant. Unused food and wastes were removed from dishes before new food was added and water was replaced with standing tap water when needed. Artificial lighting was used, with hours of light approximating as nearly as possible day-length for the particular time of year. Total and snout-vent lengths were measured at monthly intervals for three months; after this time, when growth rate increased rapidly, weekly measurements were taken. Absorption of external gills was chosen as the criterion for metamorphosis. Even though larvae in each group were initially selected at random, there was a slight difference in average size of each group. At the beginning of the experiment the average length of all the larvae was 24.7 mm. with a range of 18–33 mm. Average lengths for the individual groups at first measurements were: A, 23.9 mm.; AA, 23.6 mm.; B, 25.4 mm.; BB, 25.7 mm. Sixty-seven per cent reached metamorphosis—23 larvae in group A, 14 in AA, 13 in B, and 17 in BB. Most deaths occurred just prior to metamorphosis.

Results (Tables I and II).—The length of the larval period after animals were placed under controlled conditions was found to be shortened both with greater

TABLE I

Group averages for total lengths at metamorphosis, increase in length during laboratory observations, time to metamorphosis, and differences in these quantities between groups. Because of insufficient variation among individuals, the t *value is not given for time differences of the 20°C. groups*

Group	n	Mean length (mm.)	Mean length inc. (mm.)	Diff. (mm.)	Mean time to met. (days)	Diff.	*t*	d.f.
A	23	57.7	33.4	9.4	114	14	8.3	35
AA	14	47.6	24.0		128			
B	13	58.1	32.6	7.9	82	9	—	—
BB	17	50.5	24.7		91			

TABLE II

The significance of effect of quantity of food on mean length of larvae at metamorphosis, t value, and degrees of freedom

Group	Diff. in mean lengths (mm.)	*t* value	d.f.	significance
A* AA	10.1	9.5	35	+++
B* BB	7.6	4.1	28	+
A* BB	7.2	8.8	38	+++
B* AA	10.4	7.9	25	+++

* Longer of two groups

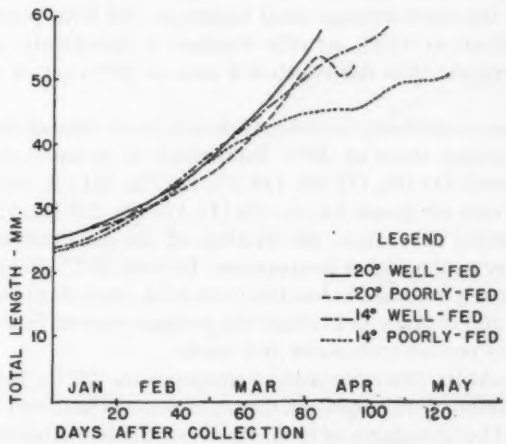

FIG. 1. Average growth rates of larvae under experimentally controlled conditions.

abundance of food and with higher degree of water temperature. Of the larvae held at 14°C., the well-fed (group A) metamorphosed, on the average, 14 days earlier than the poorly-fed (group AA), which transformed after 128 days in the laboratory. This difference is highly significant ($t = 8.3$, d.f. = 35). Of the larvae held at 20°C., all the well-fed ones (group B) metamorphosed on the 82nd day; 15 of the poorly-fed animals (group BB) metamorphosed on the 90th day, the other two, three days later (average, 91 days). This difference is again highly significant. (Because there was almost no variation in time to metamorphosis between individuals within these two latter groups, the t test would not apply here.)

The difference in water temperature seemed to affect time to metamorphosis

even more than amount of feeding. Well-fed larvae (B) at 20°C. metamorphosed 32 days before similarly fed larvae (A) at 14°C.; poorly-fed larvae at 20°C. (BB) metamorphosed 37 days before those at 14°C. (AA). These results indicate that both abundance of food and higher temperature independently hasten metamorphosis. Since amount of food consumption in poikilothermic animals is largely determined by temperature, it is not possible from this experiment to say which actually produces the greater effect.

Within each group, there proved to be no correlation between length attained at metamorphosis and time to metamorphosis (group A, $r = 0.1$, d.f. = 21; group AA, $r = 0.4$, d.f. = 12). Of the 67 surviving larvae, the range in total length at metamorphosis was from 41 to 63 mm., and in time to metamorphosis, from 82 to 132 days. In these respects, larvae held at 14°C. were more variable than those held at 20°C.

The total length at metamorphosis was found to depend on the amount of available food; at each temperature the well-fed larvae were significantly longer than the poorly-fed ones (10.1 mm. longer at 14°C. and 7.6 mm. at 20°C.). However, the difference of six degrees C. did not appear to have any effect. Both well-fed groups had the same average total length, as did both poorly-fed groups. Well-fed individuals at 14°C. actually attained a significantly greater length before metamorphosis than did poorly-fed ones at 20°C. (cf. A vs. BB, Table II).

The time to metamorphosis varied considerably more among the animals kept at 14°C. than among those at 20°C. Individuals in group A metamorphosed after 107 (4 larvae), 111 (5), 115 (8), 118 (1), 120 (1), 124 (2), and 126 (2) days; the comparable data for group AA are 124 (1), 126 (2), 127 (1), 129 (6), 130 (2), 131 (1), and 132 (1) days. Also, the duration of the metamorphic process was somewhat shorter at the higher temperature. In both 20°C. groups (B, BB) the gills were completely resorbed in less than one week after shortening of the gills was first noted, and in many individuals the process occurred in 2–3 days. In the 14°C. groups this process took about two weeks.

Conclusions.—At the two experimental temperatures (20°C., 14°C.) and under the feeding conditions here employed, metamorphosis is hastened both by higher temperature and by abundance of food. However, the size attained before metamorphosis depends on the amount of available food but not on the temperature difference. The results suggest that, at the higher temperature, the length of the larval period is less variable, and the metamorphic process is more rapid.

FIELD OBSERVATIONS.—Populations from the two natural habitats, called "Mann's Chapel Pond" and "Salamander Pool", were sampled at monthly intervals during the 1947–1948 and 1949–1950 seasons. The larvae were captured in hand nets, killed in weak alcohol, measured, and transferred to 80% alcohol. Stomachs were removed from preserved animals and their contents examined for gross identification. The 330 larvae ranged in length from 20 to 57.5 mm. Water temperatures were recorded at the time of collection.

The two ponds exhibited marked ecological differences. Mann's Chapel Pond, as compared with Salamander Pool, is much warmer because it is exposed

to direct sunlight, it covers a much larger area and it is deeper. It has a much greater abundance of aquatic vegetation and invertebrates to provide oxygen, shelter, and food for its less crowded salamander population.

Larvae in Mann's Chapel Pond metamorphosed much sooner and at a larger size than those from Salamander Pool. At each of the last spring collections made, larvae showed gill reduction or had completely lost their gills. Larvae were metamorphosing in Mann's Chapel Pond at least 49 days earlier in 1948, and 70 days earlier in 1950, than in Salamander Pool (Fig. 2; Table III). The average larval length at that time was correspondingly longer in Mann's Chapel Pond than in Salamander Pool (7 mm. longer in 1948; 8 mm. longer in 1950). The rate of growth also was much more rapid in Mann's Chapel Pond than that in Salamander Pool during both seasons studied.

A description of the two habitats will explain many of the differences between larvae growing in them. Mann's Chapel Pond lies in an open grass meadow located in a comparatively flat region. There are no trees in the vicinity of the pond; the whole area is so crowded with small shrubs and herbaceous vegetation

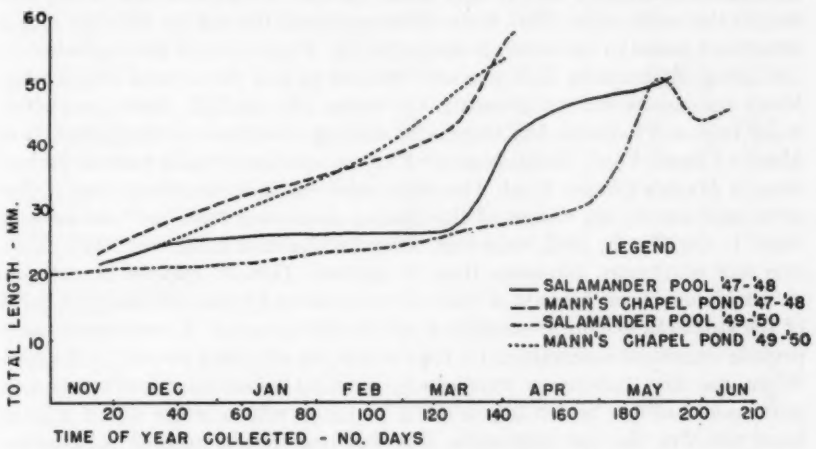

FIG. 2. Average growth rates of larvae collected from natural habitats.

TABLE III

Comparison of approximate date of metamorphosis, mean length of metamorphosing larvae, and recorded temperatures of the natural habitats

Year	Locality	Approx. date of metam.	Diff. (days)	Mean length (mm.)	Diff. (mm.)	Mean temp. (°C.)	Diff.
1947–48	Mann's Chapel Pond	April 4	49	57.5	7.1	14.3	6.3
	Salamander Pool	May 23		50.4		8.0	
1949–50	Mann's Chapel Pond	March 31	70	53.4	8.4	21.4	11.7
	Salamander Pool	June 9		45.0		9.7	

—grasses, clumps of rushes, large mats of submerged and emergent aquatic vegetation—that at a short distance the pond gives the impression of being a shallow marsh. The depth and size of the pond vary considerably, depending largely on rainfall. In the winter it covers approximately an acre, reaching a depth of two to three feet in the central portion. By June the water is reduced to half its normal depth and the area becomes only a marsh by late summer. At all times, the water is crowded with myriads of protozoa, entomostracans, insect nymphs and larvae, and water bugs. Adult *Diemictylus viridescens* was the dominant vertebrate inhabitant of the pond; also common were tadpoles of *Rana clamitans* and *Rana pipiens*.

Salamander Pool is located at the foot of a rather steep, north-facing slope in a thickly wooded area. It is well shaded by the surrounding growth of bushes, trees, and dense undergrowth in the late spring and summer, and by the slope in the winter. It was found covered with ice in mid-afternoon for the January 1950 collection. There is little or no aquatic vegetation present, but the pool is filled with debris and decaying leaves from surrounding growth. The pool, much smaller than Mann's Chapel Pond, is only about 15 feet in diameter, and 1–1½ feet deep in the center when filled. Some water is present throughout the year except for a short period in late summer and early fall. At the time of final collection in the spring, Salamander Pool was also reduced to half the normal winter area. Many entomostracans are present in the water, also crayfish, worms, and a few water bugs and tadpoles, but there is no striking abundance of life as there is in Mann's Chapel Pond. In Salamander Pool, *A. opacum* is much more abundant than in Mann's Chapel Pond. The other most common amphibian was *Ambystoma maculatum*; egg masses of this species containing "tail-bud" larvae were found in the March, 1950, collection. They had hatched before the April collection and were more numerous than *A. opacum*. That *A. opacum* larvae used *A. maculatum* larvae for part of their diet was shown by stomach analyses, 9.7% of the stomachs examined containing salamander remains. *A. maculatum* could provide important competition for the not too plentiful food present in the pool. When the first collections were made, marbled salamander larvae were so numerous that five to ten larvae could be taken with a single dip of a small hand net. For the last collections, this was true for the spotted salamander, whereas several trials had to be made repeatedly in different areas of the pool before a single *A. opacum* larva could be found. Tadpoles of *Rana pipiens* and *Pseudacris nigrita nigrita* were present in Salamander Pool, but anuran tadpoles would not compete for food since they are herbivorous, whereas salamander larvae feed principally on moving organisms.

The considerably higher water temperatures of Mann's Chapel Pond are shown by averages obtained from monthly readings from the two habitats (Table III). Readings were taken on the same day, one-half hour apart, from November through April. The recorded temperature range for Mann's Chapel Pond during the observed period was 9–27.3°C.; for Salamander Pool, 2.2–19°C.

Results of stomach analyses are shown in Table IV. Approximately 35 kinds of

TABLE IV

Stomach contents of 330 marbled salamander larvae taken from two natural habitats during the 1947–48 and 1949–50 seasons

Food item	Salamander Pool 226 stomachs		Mann's Chapel Pond 104 stomachs	
	total number	percent of stomachs	total number	percent of stomachs
Algae				
One-celled	abundant	1.3	—	—
Filamentous	—	—	2	1.9
Docidium	1	0.4	—	—
Volvox	—	—	38	10.4
Plant seeds	—	—	2	1.0
Protozoa				
Arcella	—	—	1	1.0
Rotifer	—	—	1	1.0
Oligochaeta				
Stylaria	1	0.4	—	—
Unidentified	3	1.3	1	1.0
Cladocera				
Bosmina	219	13.5	1169	32.7
Daphnia	1889	35.5	168	51.9
Unidentified	460	11.9	529	21.2
Ostracoda				
Cypris	366	10.6	49	15.4
Unidentified	1341	54.9	13	8.7
Copepoda				
Calanoid	11	2.2	—	—
Cyclopoid	1527	68.1	637	53.9
Harpacticoid	203	25.7	80	18.3
Unidentified	226	7.5	58	12.5
Isopoda				
Asellus	14	4.0	29	8.7
Amphipoda	61	6.2	—	—
Collembola	—	—	2	1.0
Odonata				
Damselfly nymphs	—	—	7	4.8
Dragonfly nymphs	—	—	4	3.0
Ephemerida				
Mayfly nymphs	—	—	76	23.1
Hemiptera				
Water bug	2	0.9	1	1.0
Coleoptera				
Beetle larvae	106	20.8	27	13.5
Diptera				
Mosquito adult	1	0.4	—	—
Mosquito larva	2	0.9	3	3.0
Unidentified larva	3	1.3	—	—
Unidentified pupa	1	0.4	—	—
Insect larva				
Unidentified	13	4.8	11	4.8
Acarina				
Hydracarina	—	—	5	4.8
Gastropoda				
Planorbis	5	0.9	44	21.2
Amphibia				
Salamander gills	14	5.7	2	1.0
Salamander tails	3	1.3	—	—
A. maculatum larvae	8	2.7	—	—
Frog eggs	32	3.3	1	1.0
Miscellaneous	1	0.4	—	—

food were found. The most abundant forms in both ponds were entomostracans, but there were considerable differences in food types utilized by larvae from the two habitats. Those forms requiring abundant sunlight and aquatic vegetation were absent from Salamander Pool, which afforded a smaller variety of food organisms than was found in larvae from Mann's Chapel Pond. This is probably evidence of faunal differences between the two habitats rather than any selective preference on the part of the larvae. Plant material in the stomachs may have been accidentally ingested. Stomachs of larvae metamorphosing at the time of collection were empty, or contained only undigested shells of crustaceans and molluscs.

DISCUSSION.—Of the two natural habitats, Mann's Chapel Pond exhibited higher temperature and more abundance as well as a greater variety of food. Its larvae were found to metamorphose earlier and at a larger size. This agreement with laboratory results seems to imply that both the higher temperature and better food conditions hastened metamorphosis in Mann's Chapel Pond whereas the second factor was more important in increasing size at metamorphosis.

Several factors not operating in the laboratory which could influence development in the natural habitats should be mentioned. Drying of the habitat is often a stimulus to amphibian metamorphosis (Dempster, 1930). Both ponds were much reduced in size at the time of final collection, this time coming much sooner for Mann's Chapel Pond than for Salamander Pool. The greater amount of light on the Mann's Chapel habitat may have had a direct effect on animal development by influencing the thyroid gland (Rugh, 1935; D'Angelo *et al.*, 1941; Stein and Carpenter, 1943; Doetsch, 1949). Increased light would also stimulate growth of aquatic vegetation, thereby increasing oxygen content of the water. Mann's Chapel Pond afforded not only a larger quantity of food, but also a greater variety. Competition for food was not so great as in Salamander Pool where many more animals had to live on a relatively scarce food supply. The size of probable food items changes in the spring when insect nymphs, so numerous in Mann's Chapel Pond, grow larger. Crowding may have had an inhibitory effect on growth of larvae in Salamander Pool (Rugh, 1934; Hutchinson and Hewitt, 1935).

In spite of these complicating factors in the natural habitats, measurements from the 14°C. poorly-fed and the 20°C. well-fed experimental groups correspond quite closely, both in length at metamorphosis and in duration of larval period, to measurements of larvae from Salamander Pool and Mann's Chapel Pond, respectively.

Controlled groups exhibited a much more constant growth rate than did larvae taken from natural habitats. In the latter there was no appreciable change in size until about a month prior to metamorphosis, when there was a marked length increase (cf. Fig. 1 and Fig. 2). This could have been caused by a sharp temperature rise in late spring months. Days were longer, food supply increased, and there was less competition and crowding since many larvae had succumbed by that time. In the laboratory animals, a definite drop in growth rate was observed just prior to metamorphosis. In many larvae, there was an actual reduc-

tion in length just prior to and at the time of gill resorption. Feeding slowed considerably or ceased altogether. Stomach analyses of metamorphosing animals from the natural habitats revealed similar evidence. This is in accordance with observations of previous investigators (Wilder, 1924; Dempster, 1930). Part of the length decrease could be attributed to tail shrinkage as the fin is resorbed.

At low temperatures amphibian growth rate is retarded and a longer time is required to reach metamorphosis (Lillie and Knowlton, 1898; Atlas, 1935; Ryan, 1941); this longer growth period may result in larger larvae at metamorphosis (Uhlenhuth, 1919; Morgan and Fales, 1942). According to results obtained in this study, the temperatures used did not affect size at metamorphosis although food abundance did. Uhlenhuth (1921) obtained *A. opacum* larvae approximately 10 mm. longer at metamorphosis at 15°C. than at 25°C. He does not indicate food conditions. If his larvae at 15°C. were better fed than those at 25°C., the present results would agree with his (cf. lengths of groups AA and B); otherwise, a possible explanation of his results might be that both temperatures used in this study fall close to an optimum for *A. opacum*, whereas his high of 25°C. was less favorable. Optimum range for urodele larvae is 12–25°C. (Rugh, 1948).

SUMMARY

1. One hundred young larvae of *Ambystoma opacum* Gravenhorst, collected in one random sampling from a natural population, were grown in individual dishes under the following conditions: 50 larvae at 14°C., 25 of them fed abundantly, and 25 poorly fed; 50 larvae at 20°C., 25 fed abundantly and 25 poorly fed.

2. Records were taken of growth rates, time to metamorphosis and length at metamorphosis for individuals. Averages were calculated in order to determine the effect of these differences in temperature and quantity of food.

3. At 14°C., well-fed larvae metamorphosed in 114 days, 14 days sooner than poorly-fed larvae; at 20°C., well-fed larvae metamorphosed after 82 days in captivity, 9 days sooner than poorly-fed individuals. At both temperatures, well-fed larvae metamorphosed at 57 to 58 mm. and poorly-fed larvae at 47 to 50 mm.

4. The results show that higher temperature and food abundance each hasten metamorphosis and increase the rate of growth, whereas only the second factor is apparently responsible for greater length at metamorphosis at the two particular temperatures used.

5. Collections of larvae were made from two natural habitats differing in environmental conditions, especially in temperature and available food. Differences found in growth rate, size at metamorphosis, and length of larval period may be interpreted from the results of the laboratory observations. The greater size of larvae in the warmer, sun-lit natural habitat was interpreted to be more the result of abundant food supply than of higher temperature. Earlier time of metamorphosis and faster growth rate in the warmer habitat are apparently brought about by both greater food abundance and higher temperature.

6. Stomach analyses were made of 330 larvae collected over a period of two

years from the natural habitats studied. Approximately 35 different kinds of food items were found, the most abundant forms being cladocera, ostracods, and copepods. Food differences noted between the two habitats seemed to reflect types of available food rather than any preference on the part of the larvae.

LITERATURE CITED

ATLAS, MEYER. 1935. The effect of temperature on the development of *Rana pipiens*. Physiol. Zool. **8**: 290–310.

D'ANGELO, S. A., A. S. GORDON AND H. A. CHARIPPER. 1941. The role of the thyroid and pituitary glands in the anomalous effect of inanition of amphibian metamorphosis. Jour. Exp. Zool. **87**: 259–275.

DEMPSTER, W. T. 1930. The growth of larvae of *Ambystoma maculatum* under natural conditions. Biol. Bull. **58**: 182–192.

DOETSCH, H. 1949. Experimentelle Untersuchunger über den Einfluss des Lichtes und der ultravioletten Strahlen auf Wachstum und Entwicklung von Amphibienlarven. Arch. Entw. Mech. Org. **144**: 25–30.

HUTCHINSON, C., AND DOROTHY HEWITT. 1935. A study of larval growth in *Ambystoma punctatum* and *Ambystoma tigrinum*. Jour. Exp. Zool. **71**: 465–482.

LILLIE, F. E., AND F. P. KNOWLTON. 1898. The effect of temperature on the development of animals. Zool. Bull. **1**: 179–193.

MORGAN, A. H., AND C. H. FALES. 1942. Seasonal conditions and effects of low temperature in the thyroid glands of amphibians. Jour. Morphol. **71**: 357–390.

NOBLE, G. K., AND M. K. BRADY. 1933. Observations on the life history of the marbled salamander, *Ambystoma opacum* Gravenhorst. Zoologica **11**: 89–132.

RYAN, F. J. 1941. Temperature change and the subsequent rate of development. Jour. Exp. Zool. **88**: 25–54.

RUGH, ROBERTS. 1934. The space factor in the growth rate of tadpoles. Ecology **15**: 407–411.

———. 1935. The spectral effect on the growth rate of tadpoles. Physiol. Zool. **8**: 186–195.

———. 1948. Experimental Embryology. Minneapolis: Burgess Publishing Company.

STEIN, K. F., AND K. CARPENTER. 1943. The effect of increased and decreased light on the thyroid gland of *Triturus viridescens*. Jour. Morph. **72**: 491–517.

UHLENHUTH, EDUARD. 1919. Relation between thyroid gland, metamorphosis, and growth. Jour. Gen. Physiol. **1**: 473–482.

———. 1921. The internal secretions in growth and development of amphibians. Amer. Natur. **55**: 193–221.

WILDER, INEZ W. 1924. The relation of growth to metamorphosis in *Eurycea bislineata*. Jour. Exp. Zool. **40**: 1–112.

BIOLOGY AND PROPAGATION OF YOUNG HARD CLAMS, *MERCENARIA MERCENARIA*[1]

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The hard clam, *Mercenaria* (=*Venus*) *mercenaria* L., is known popularly as a nutritional delicacy and is available principally along the eastern and gulf coasts of the United States. It is harvested almost entirely as a wild crop. Young clams are available in dense concentrations only in a few coves on the Maine coast. Sufficient technical information is not available to permit propagation of the bivalve on a large scale. Because of increasing demands for food products of coastal areas this estuarine species has been the subject of considerable study in the last few years.

The author (in cooperation with T. C. Nelson and H. H. Haskin) began studies on the field culture, gross structure, behavior, and ecology of the early stages of the hard clam in New Jersey[2] in 1947; and in 1952 (in cooperation with J. R. Nelson, H. N. Gibbs, M. C. Gale, V. L. Loosanoff, and H. Davis) commenced a preliminary three-year investigation on the utilization of Home Pond, a salt pond on Gardiners Island, New York, for possible large scale propagation of the hard clam and other bivalves.

In brief, this eight-year investigation has involved: (1) study of the biology, particularly of the early more vulnerable stages, of the clam; (2) development of methods of culturing young clams *en masse* in field laboratories and in salt ponds; (3) and development of means of protecting newly set clams in the field, where they are easy prey to certain crabs and gastropods.

The results of these studies may be summarized as follows: Morphology and behavior of young clams change markedly as they grow older. About 24 hours after fertilization of the discharged ova, embryos develop into fully-shelled free-swimming veligers with a ciliated swimming organ, the velum. These remain in schools, and as planktonic animals float passively with the moving tidal water masses. Minor vertical peaks of larval concentration occur about one meter below the surface. In approximately 7 days the veliger develops a foot and becomes a pedoveliger. The foot is extended by the swimming larva as it approaches the substratum; once contact is effected it crawls over the substratum, then may swim off to a different site, alight, and creep further. This behavior may be useful in locating favorable bedding surfaces and is displayed for several days. During the searching period a byssus gland develops in the heel of the foot and the clam commences weak byssal (a slender thread) attachment to the

[1] This paper is a condensation of an illustrated lecture given before the Elisha Mitchell Scientific Society in Chapel Hill, October 11, 1955.

[2] The studies in New Jersey were sponsored jointly by Rutgers University and the Campbell Soup Company of New Jersey; those on Gardiners Island, at first jointly by the J. & J. W. Elsworth Company and the Monarch Buick Company, Inc. (M. C. Gale, owner) of New York, and later jointly by the U. S. Fish and Wildlife Service and the Monarch Buick Company, Inc.

substratum. Soon the velum is lost and the clam, now a crawler in the byssal stage, for a number of weeks alternates between byssal attachment and crawling. Byssal attachment tends to hold the clam at its original setting site, thereby reducing the likelihood of transport by tidal currents. When the clam reaches a length of approximately 7 mm. the byssus gland becomes functionless, and the bivalve, now a juvenile, maintains its position in the bottom solely by means of its foot.

When the pedoveliger settles to the bottom its mantle edges are unfused, and the clam positions itself at the surface of the bottom sediment. Shortly, however, a portion of the mantle margins fuse, and a thin excurrent siphonal sleeve develops in the posterior opening thus formed. Siphonal tentacles emerge from the mantle edges and border the excurrent siphon which is later projected with the sleeve on its distal end. The incurrent siphon develops later, also with tentacles, but lacks the sleeve. Finally the excurrent sleeve is resorbed and the siphons assume a definitive form. As the siphons lengthen the juvenile clam is able to burrow deeper into the bottom, and by the time the definitive siphons form, it has completely buried itself in the sediment, thereby receiving partial protection from predators.

Guided by the methods of V. L. Loosanoff and associates, we made numerous attempts to culture clam larvae in large quantities in field laboratories. Mature clams were spawned artificially and clam larvae developing from artificially fertilized ova were maintained in large containers of still sea water. Water was changed every other day and a mixed culture of *Chlorella* was fed to the larvae. This was grown by fertilizing sea water, inoculated with *Chlorella*, by the addition of an organic fertilizer such as 5–3–5. Larvae were also grown to the pedoveliger stage on minute quantities of extracts of cereal pablum, presumably feeding on bacteria and other micro-organisms stimulated to rapid growth by this extract. In both cases they reached the pedoveliger stage in 10 to 14 days. Why, in native waters, larvae develop to this stage in as brief a period as 7 days is not known. In the course of 7 summers only occasional cultures of clam larvae reached the byssal stage in large numbers. Much of the cause of this high mortality rate is attributed to a fungus, *Sirolpidium*, described first by H. C. Davis and associates, which destroyed a part or all of many of our cultures. Other causes of mortality are unknown.

These repeated failures emphasized the need of circumventing the expensive, laborious, and our generally unreliable field laboratory culture of larvae. The utilization of partly enclosed natural salt ponds suggested itself for raising both clam larvae and juveniles. These ponds provide protection from excessive wave action and tidal currents, readier control of enemies, easy access to the bottom for planting, harvesting, and management of the clams, ease of construction and maintenance of barriers to enemies, and make possible construction of gates across the inlet to impound water and, most important, to retain larvae during their planktonic stages. There is the further advantage that the impounded waters may be fertilized to accelerate growth of food organisms if these are present in insufficient quantities.

Accordingly three summers were devoted to a study of the hydrography and biology of Home Pond. Emphasis was placed on a study of relatively undisturbed conditions preparatory to future studies in which the inlet would be closed temporarily. The pond measures 3,000 feet in length and averages 300 feet in width. The inlet has a width of about 30 feet and an average depth of two feet at mean low water. The pond is relatively shallow, sloping gently from low steep banks to a depth of 2½ feet at mean low water. The normal tidal amplitude varies from 0.9 to 2.2 feet, so that the third of the bottom of the pond which consists of firm sand or sandy mud is exposed or under only a few inches of water at low tide. The remainder of the pond bottom consists of fine black mud which occurs in layers ranging in depth from a thin layer to over 14 feet in a few places. Inlet sediments consist of coarse gravel, sand, and shell. About 50 to 70% of the water in the pond moves out into the bay during an average ebbing tide. Maximum current velocities occur at the inlet where velocities of 115 cm./sec. occur repeatedly. Because of the pattern of circulation of water in Gardiners Bay off Home Pond, water ebbing from Home Pond rarely returns to the pond on the following flood tide. A sill at the inlet retains water in the pond at low water. The pond fills in about four hours and empties in about eight hours. The shallow waters of the pond warm readily during the summer and temperatures as high as 33.4°C have been recorded from the upper reaches. Because of wind action and the shallowness of the pond very little vertical thermal stratification occurs. The only fresh water entering the pond enters as seepage from the surrounding forested slopes. Salinity during the summer ranges from 22 to 30‰, and because there is relatively little fresh water seepage, falls below 28‰ only during periods of heavy rainfall. Fresh water tends to pool on the surface in thin layers, soon mixing with the underlying sea water to be exchanged for that in Gardiners Bay during subsequent tides. The pH of the water, from the surface to the bottom, remains consistently between 7.8 and 8.0 during daylight hours.

Plankton in the pond is especially rich in diatoms, flagellates, copepods, worm larvae, barnacle larvae, and, during June and July, bivalve and gastropod larvae. The banks of the pond are almost entirely composed of an irregular mat of marsh grass and ribbed mussels overlying peat. Oysters of various ages grow on the mussels, but the main oyster population, the most conspicuous permanent residents, occur over the firmer portions of the submerged bottom. Oysters grow rapidly here and have attained maximum lengths of 9 to 10 inches. Hard clams also occur in moderate numbers, particularly in the vicinity of the inlet. In the upper portions of the pond they have attained maximum lengths of about 5½ inches. Thus conditions in the pond have been favorable for these bivalves for a number of years. Large numbers of burrowing worms, mud snails, mud crabs, hermit crabs, green crabs, blue crabs, and some scallops, surf clams, and razor clams also occupy the area.

Large sets of oysters and clams occur only infrequently in the pond. The rate of exchange of the water with that in the bay is so great that larvae spawned in Home Pond are flushed into Gardiners Bay before setting time is reached. Only during special and infrequent circumstances when ready-to-set larvae enter the

pond from the bay in large numbers do extensive sets occur within the pond, and these cannot be reliably predicted. This suggests the desirability of closing off the inlet at the start of the spawning season to impound the water until clam and oyster larvae set. The inlet may then be opened again to provide the circulation which is vital in preserving good growing conditions in the pond.

Best growth of hard clams was found in the inlet where in two years and three months maximal length attained ranged from 6 to 7 cm. In this same period oysters setting in the lower reaches of the pond attained maximal lengths of 10 to 11 cm.

Investigations in the pond soon demonstrated that young clams therein are subject to excessive predation from mud, green, and blue crabs. Very few newly-set clams planted in large numbers without protection survived. As clams grow larger thicker shells and bury themselves in the bottom, they become less vulnerable to attack. Of 720 clams ranging in length from 10–40 mm. when planted without protection, 76 % survived; and of 400 clams ranging in length from 35–58 mm., 91 % survived. Very poor survival was obtained by planting unprotected small clams in mud or sandy bottom, whereas as many as 40 % survived when planted in a coarse sand-gravel bottom in the swift water of the inlet. Crab predators readily dig clams, and particularly the small ones, out of the sandy and muddy bottoms, whereas in sand-gravel bottoms in swift currents predation is impeded.

A substantial number of plantings of various size groups of hard clams in screened upright enclosures in Home Pond illustrated the greater protection afforded small clams as the mesh size of the screen decreases. In spite of such protection, however, maximum survival attained was only 88 %. Small crabs, particularly mud crabs, crept through the screen, moulted, and could not escape. From a consideration of construction and maintenance of enclosures of various mesh sizes, exclusion of enemies, and flow of tidal water through the enclosures, our information indicates that fenced enclosures of ¼ inch mesh, painted with antifouling paint, are most satisfactory for protecting small hard clams. In spite of such enclosures it is necessary to search periodically within the enclosures for crabs which have grown too large to leave. By using wooden floats screened with ½ inch mesh screen in which small clams, either in or without sand, were stationed a few inches below the surface of the water, survival of 99–100 % was obtained during the course of one summer. Mechanical enclosures of this kind, though the best available at the time, are probably not the best means of barring enemies; some means of chemical control such as several being actively investigated by V. L. Loosanoff and his associates may prove more effective.

These preliminary researches indicate a few of the avenues for further research required before large scale propagation of hard clams in salt ponds is possible. Primary among these is the temporary closure of inlets to prevent the flushing of larvae from ponds. This approach, though it may require the augmentation of the larval food supply in the pond, would circumvent the expensive and often unreliable laboratory method of rearing clam larvae in large numbers for planting in ponds.

A NEW CRAYFISH OF THE GENUS CAMBARUS FROM NORTH CAROLINA AND SOUTH CAROLINA (DECAPODA, ASTACIDAE)

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Although specimens of this previously undescribed species have been in my collection for approximately five years, the desire to have available more than a single first form male and to secure additional locality records has delayed this report.

In several respects this species seems to be the eastern counterpart of *Cambarus striatus* Hay (1902: 437), which is found in Alabama, Tennessee, and Kentucky. As in this species the narrow areola constitutes 37 to 41 per cent of the total length of the carapace. The habits of neither species are adequately known but both have been reported from small streams and burrows. In only one locality has the new species been collected in abundance but evidence of a colony of some size was observed in a poorly drained seepage area at Rockwell in Rowan County, N. C. Here, more than a score of burrows were counted in an area of approximately 800 square feet.

I wish to express may appreciation to the following persons who have aided me in securing the specimens on which the following description is based: Dr. David G. Frey, Dr. Eugene N. Kozloff, Mr. Edward A. Crawford, Mr. Thomas R. Bello, and Mr. Kenneth W. Simonds.

Cambarus reduncus[1], sp. nov.

DIAGNOSIS.—Rostrum acuminate, distinctly excavate, and without lateral spines at base of acumen. Areola 9.4–28.4 times longer than broad and comprising 37.1–41.8 per cent of entire length of carapace. Lateral surfaces of carapace with one or two small tubercles immediately caudal to cervical groove. Suborbital angle absent. Postorbital ridges with or without a small tubercle. Antennal scale less than one-half as broad as long. Chela with two rows of tubercles along inner margin of palm; outer margin of hand costate and both fingers with well-defined longitudinal ridges on upper surfaces. First pleopod (Figs. 1, 3) of first form male with the slender, tapering central projection in the form of a broad arc the distal end of which lies at a level proximad of its base. Annulus ventralis (Fig. 4) slightly movable and deeply situated between the other sternal sclerites.

HOLOTYPIC MALE, FORM I.—Body subovate, compressed laterally. Abdomen narrower than thorax (14.7 and 17.6 mm. in widest parts respectively). Greatest width of carapace greater than depth in caudodorsal margin of cervical groove (17.6 and 16.4 mm.). Areola narrow (17.2 times longer than wide), with few punctations, only two across narrowest portion. Cephalic section of carapace 1.4 times as long as areola (length of areola 41.8 per cent of entire length of carapace). Rostrum deeply excavate above with convergent, only slightly raised, margins; acumen not distinctly delineated but apex with a small, up-turned, corneous

[1] *reduncus:* L., curved or bent backward; referring to the greatly curved central projection of the first pleopod of the first form male.

tubercle; lateral spines or tubercles absent. Punctations on upper surface sparsely distributed except in submarginal row. Subrostral ridges moderately well developed and visible in dorsal aspect slightly beyond caudal half of rostrum.

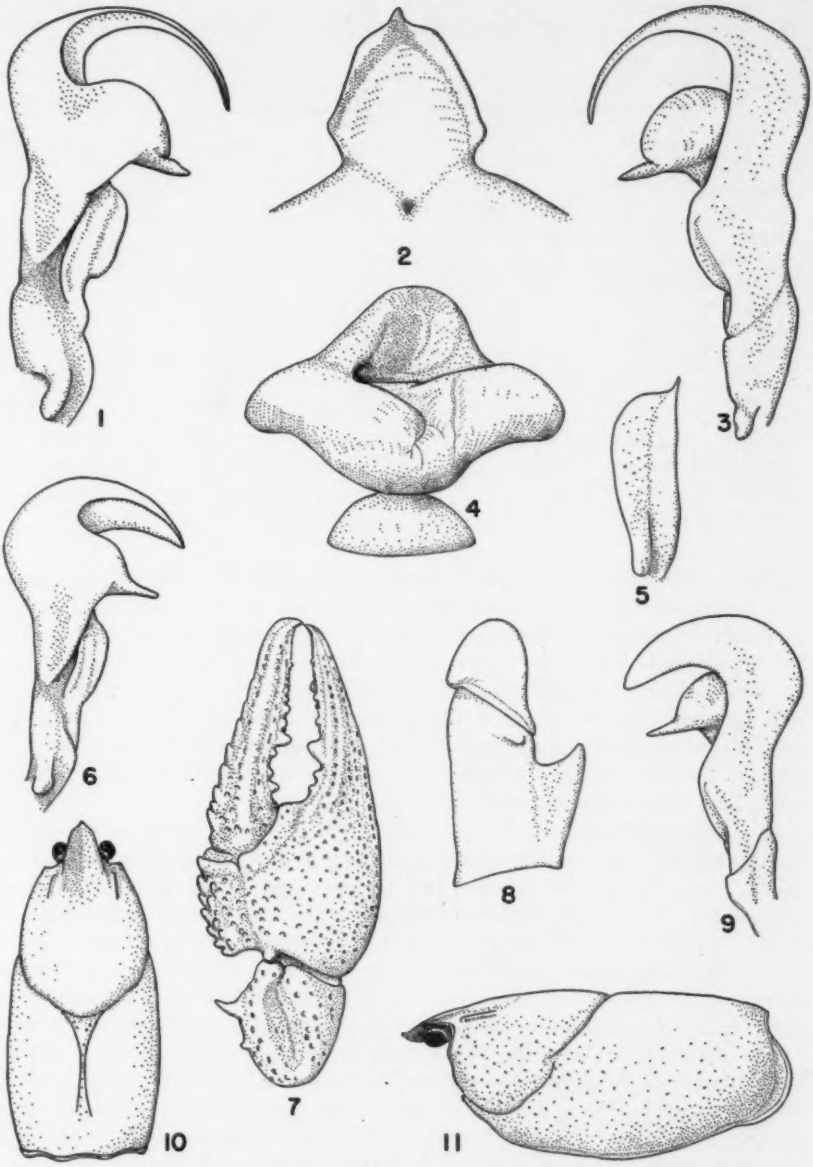
Postorbital ridges moderately well developed, with shallow lateral grooves, and terminating cephalad in small tubercles. Suborbital angle obsolete. Branchiostegal spine small and poorly defined. No spines or prominent tubercles on side of carapace immediately caudad of cervical groove. Carapace punctate dorsad and somewhat granulate laterad, particularly so in area cephalic to cervical groove. Abdomen shorter than carapace (33.1 and 36.9 mm.). Cephalic section of telson with two spines in each caudolateral corner.

Epistome (Fig. 2) subequal in length and breadth with a slightly convex surface; margins distinctly elevated (ventrally) and cephalic extremity with a prominent cephalomedian projection. Antennules of the usual form with a small spine on the lower surface of the basal segment. Antenna extending caudad to third abdominal segment. Antennal scale (Fig. 5) broadest in penultimate fourth, but not conspicuously so; mostly narrow and subrectangular with lamellar portion only slightly narrower than outer heavier area, the latter terminating in a well developed acute spine.

Right chela strongly depressed but with the palm inflated; distal two-thirds of outer margin of hand costate. Upper surface of palmar area with squamous tubercles mesially and punctations laterally. Inner margin of hand with two rows of tubercles, the more mesial one consisting of seven and the more lateral one of only five. Lower surface of palmar area similar to upper surface but punctations shallower and tubercles smaller; but four conspicuous tubercles present—two on ridge at base of dactyl and two slightly proximad of them. Fingers slightly gaping; upper and lower surfaces of both with well-defined longitudinal ridges subtended by setiferous punctations. Opposable margin of immovable finger with seven tubercles, the third and sixth the larger. Opposable margin of dactyl with ten tubercles, the first and fourth larger. Distal half of opposable margins of both fingers with a single row of minute denticles interrupted by the more distal tubercles mentioned above. Mesial margin of dactyl with a row of eight tubercles, progressively more squamous distally; proximal portion of row flanked by other tubercles and, distad of the row, a linear arrangement of four setiferous punctations.

Carpus of right cheliped distinctly longer than broad, and with a distinct longitudinal furrow above; except for mesial and mesioventral surfaces, entire podomere punctate. Mesial surface with one prominent procurved spine slightly distad of midlength and a smaller one proximad of it; mesioventral surface with two inconspicuous tubercles opposite the larger one on mesial surface. Distal ventral margin with the usual two tubercles.

Merus of right cheliped sparsely punctate mesially and laterally. Upper surface with two prominent tubercles near distal margin subtended by several inconspicuous ones. Lower lateral margin with a row of five tubercles and inner lateral margin with a row of 11. Both rows well defined with only one tubercle lying between the two. Lower mesial row of tubercles on merus with a corresponding row on ischiopodite consisting of six tubercles.



FIGS. 1–11. *Cambarus reduncus*, sp. nov. FIG. 1. Mesial view of first pleopod of first form male. FIG. 2. Epistome. FIG. 3. Lateral view of first pleopod of first form male. FIG. 4. Annulus ventralis of female. FIG. 5. Antennal scale of male. FIG. 6. Mesial view of first pleopod of second form male. FIG. 7. Upper surface of cheliped of first form male. FIG. 8. Basipodite and ischiopodite of third pereiopod of first form male. FIG. 9. Lateral view of first pleopod of second form male. FIG. 10. Dorsal view of carapace of first form male. FIG. 11. Lateral view of carapace of first form male.

TABLE I

Some measurements of type specimens of Cambarus reduncus (in mm.)

Structure measured	Holotype ♂	Allotype ♀	Morphotype ♂
Carapace:			
Height	16.4	15.6	12.6
Width	17.6	17.3	14.6
Length	36.9	35.6	29.1
Areola:			
Width	0.9	0.5	0.4
Length	15.5	14.2	11.4
Rostrum:			
Width	4.7	5.1	4.5
Length	6.4	6.5	5.4
Chela:			
Length of inner margin of palm	7.6	7.4	5.3
Width of palm	13.2	11.7	8.8
Length of outer margin of hand	26.0	24.8	18.1
Length of dactyl	18.4	16.7	12.9

Hooks on ischiopodites of third pereiopods only (Fig. 8); hooks strong, simple and not opposed by a tubercle on basipodite. Coxa of fourth pereiopod with a prominent caudomesial protuberance; that of fifth pereiopod with no ventrally projecting prominence.

Sternum deeply situated between bases of third, fourth, and fifth pereiopods and a cephalically-converging setiferous brush lying mesiad of bases of third and fourth pereiopods. The deep sternum and the conspicuous brush would serve to protect the delicate greatly elongated central projection of the first pleopod when held against the sternum.

First pleopod (Figs. 1, 3) symmetrically arranged and reaching coxopodite of third pereiopod when abdomen is flexed; distal portion terminating in two distinct parts. Central projection corneous, very long, strongly recurved, and tapering to a tip which lies proximad of its base. Mesial process non-corneous, bulbiform with a small finger-like apical projection, and directed at an angle of about 50° to main longitudinal axis of appendage; thus, both terminals directed caudad and somewhat proximad. Oblique groove (Fig. 3) marking basipidoischial articulation more prominent than in first form males of most species.

MORPHOTYPIC MALE, FORM II.—Except for differences in size (see Table I), slight variations in tubercle counts, and in the secondary sexual characteristics, the morphotypic male shows few variations. The right cheliped is slightly atypical and has 10 tubercles along the opposable margin of the immovable finger and 11 on the corresponding margin of the dactyl; the left cheliped bears seven and eight in the two rows respectively. The hooks on the ischiopodites of the third pereiopods are decidedly smaller and less clearly defined; the brush of setae on the sternites of the segments bearing the third and fourth pereiopods is conspicuous and almost as well-marked as in the first form male. The first pleopod (Figs. 6, 9) has neither terminal element corneous and both are directed at approximately a

right angle to the main shaft of the appendage. The groove marking the articulation between the basipodite and endopodite (see Hart, 1952: 43) is more clearly defined here than in the holotype.

ALLOTYPIC FEMALE.—The allotype differs from the holotype in the following respects: The antennae extend caudad to the fourth abdominal segment. The chela is similar except the opposable margin of the immovable finger has eight tubercles; the upper row of tubercles along the inner margin of the palm consists of seven; the proximal tubercle on the mesial margin of the carpus is obsolete as are the two on the lower mesial surface; the lateral and mesial rows of tubercles on the lower surface of the merus consist of four and 10 respectively on both the right and left appendages. The annulus ventralis is deeply situated between the fourth and fifth pereiopods, and is slightly movable. Its caudal wall is very prominent, inflated, and cut by a well-marked sinus which extends dextrad just cephalic to the high dextrocaudal wall where it disappears into the fossa. The horizontal tongue extends from the cephalic surface of the sinistrocaudal wall and projects dextrally along the cephalic side of the fossa. The cephalic half of the annulus is much less conspicuous than the caudal half and bears a median longitudinal depression delimited by the higher cephalic and lateral marginal walls, and by the caudally situated tongue.

TYPE LOCALITY.—Flood plain pools of Little River, 10.3 mi. W. of Winnsboro, Fairfield Co., S.C., on Rte. 22. The pools were about 5–15 ft. in width with clay banks and bottom, and the water was so turbid that one could see the bottom only at a depth of less than six or eight inches. Some of the pools were as much as three feet in depth, and at the time the collections were made the water temperature was 20°C. Except for grasses around the edges of the pools there was no obvious vegetation in them. In and along the flood plain were *Liriodendron tulipifera*, *Liquidambar styraciflua*, *Ulmus* sp., *Quercus* sp., and *Pinus* sp. All the specimens were taken in a seine.

DISPOSITION OF TYPES.—The holotypic male, form I, allotypic female, and morphotypic male, form II, are deposited in the United States National Museum.[2] Of the 21 paratypes, one male, form II, and one female are in the collection of Dr. George H. Penn, Jr. at Tulane University and four males, form II, one female, three juvenile males, and 11 juvenile females are in my personal collection at the University of Virginia.

VARIATIONS.—Among the most conspicuous variations observed in this species is the relative width of the areola. Among the adult specimens from South Carolina the length of the areola is 17.0–28.5 (av. 22.9) times as long as broad whereas the specimens from North Carolina have a corresponding range of 9.4–15.4 (av. 12.8). The former measurements were made of specimens from Fairfield and Richland counties, South Carolina, and the latter of specimens from Rowan, Chatham, and Orange counties, North Carolina. This is the only variation that has been correlated with different populations. There are individual variations in relative length of the areola but none constitutes less than 37.1 per cent of the length of the carapace, and none more than 41.8 per cent (av. 39.5

[2] U.S.N.M. nos. 99183, 99184, and 99185, respectively.

per cent). The usual variations occur in the number of tubercles on the various podomeres, but except in the instances of regenerated chelipeds the range of variation is well within the limits pointed out in the descriptions above.

Coloration in this species is rather subdued, varying from a ground color of grayish olive to straw brown with nondescript markings on carapace and chelipeds. No distinctive markings were observed in any of the specimens collected.

HABITS.—The habits of *Cambarus reduncus* are poorly known, but several observations tend to support a tentative conclusion that it is not markedly different in its habits from *Cambarus d. diogenes* Girard (1852: 88). Like the latter species, it is usually found in the vicinity of streams where it constructs burrows, often with several passageways, the depth of which is apparently largely affected by the water table. That it may be independent of standing water was demonstrated by the situation in Rockwell, Rowan County, North Carolina, where burrows were abundant in a small seepage area in which water, even in a rainy season, would not stand but for a short while, and no streams were in the immediate vicinity. The fact that many of the available specimens were collected in seines indicates that the animals are by no means necessarily confined to burrows. There is no indication that one type of aquatic situation is preferred over another for they have been taken both in lentic and decidedly lotic habitats; from water that was relatively clear, and from water so laden with silt that an object held three inches below the surface could not be seen.

RELATIONSHIPS.—There seems to be little doubt that this species has its closest affinities with *Cambarus latimanus* (LeConte, 1855: 402) and its relatives, and in many respects it appears to be more closely allied to *Cambarus striatus* Hay than to any other member of the genus. It may be distinguished from all other species by the slender and greatly recurved central projection of the first pleopod of the first form male. Since *C. reduncus* has been collected in the same localities on several occasions with *C. latimanus*, the only closely related species in the Atlantic drainage system (excluding the Gulf of Mexico), there seems no reason to question the validity of its assignment to a distinct species.

SPECIMENS EXAMINED.—A total of 159 specimens have been examined from the following localities:

SOUTH CAROLINA—FAIRFIELD CO.: *4-1855-5a*, pools along Little River 17.2 mi. N.W. of Columbia on Rte. 215, 1♂ II, 2 juv. ♀♀, E. A. Crawford, T. R. Bello and Hobbs, coll.; *4-1855-6a*, Little River (Type Locality), 1♂ I, 3♂♂ II, 2♀♀, 1 juv. ♂, 4 juv. ♀♀, E.A.C., T.R.B., and H.H.H., coll. RICHLAND CO.: *4-1755-15*, stream 5 mi. N.E. of Eastover on Co. Rte. 409, 2♂♂ II, 1♀, 1 juv. ♂, 5 juv. ♀♀, E.A.C., coll.; *4-1755-12b*, stream S.E. of Wateree, E. of U.S. Rte. 601, 1 juv. ♂, E.A.C., coll. NORTH CAROLINA—ANSON CO.: *4-1755-5c*, stream 2 mi. S. of Morven on U. S. Rte. 52, 1 juv. ♀, T.R.B. and H.H.H., coll. CABARRUS CO.: *4-1553-4*, stream 0.1 mi. S.E. of Rowan-Cabarrus Co. line on St. Hy. 52, 2♂ II, 4♀♀, 49 juv. ♂♂, 46 juv. ♀♀. CHATHAM CO.: *4-1655-2a*, 9.8 mi. N. of Sanford on Rte. 501 1♂ I, 2♂♂ II, 1♀, 4 juv. ♂♂, 5 juv. ♀♀, T.R.B. and H.H.H., coll.; *4-1533-10a*, 4.6 mi. W. of Siler City on Rte. 64, 2 juv. ♂♂, Kenneth W. Simonds and H.H.H., coll. DAVIDSON CO.: *4-1553-6*, 3.7 mi. N.E. of Davidson-Rowan Co. line on Rte. 49, 1♂ II, K.W.S. and H.H.H., coll. LEE CO.: *4-1553-12a*, 1.8 mi. S. of Lee-Chatham Co. line on Rte. 421, 1 juv. ♂, K.W.S. and H.H.H., coll. ORANGE CO.: *5-1550-1*, field in Chapel Hill, 1♂ II, D. G. Frey, coll.; *4-654-1a*, burrows

along New Hope Creek, 5 mi. E. of Chapel Hill, 1♂ II, E. N. Kozloff, coll.; *5-254-2, ibid.*, 1♂ I, 1♀, 2 juv. ♀ ♀, E.N.K., coll. RANDOLPH CO.: near Ramseur on Rte. 64, 4 juv. ♀ ♀, K.W.S. and H.H.H., coll. ROWAN CO.: dug from burrows in Rockwell, 2♀ ♀, 3 juv. ♀ ♀, K.W.S. and H.H.H., coll.

LITERATURE CITED

GIRARD, C. 1852. A revision of the North American Astaci, with observations on their habits and geographical distribution. Proc. Acad. Nat. Sci. Philadelphia **6:** 87–91.

HART, C. W., JR. 1952. The exoskeleton and musculature of the appendages of the first three abdominal segments of *Cambarus longulus longulus* Girard (Decapoda, Astacidae). Va. Jour. Sci. **3:** 39–48.

HAY, W. P. 1902. Observations on the crustacean fauna of Nickajack Cave, Tennessee, and vicinity. Proc. U. S. Nat. Mus. **25:** 417–439.

LECONTE, JOHN. 1856. Descriptions of new species of Astacus from Georgia. Proc. Acad. Nat. Sci. Philadelphia **7:** 400–402.

THE CILIATE *DILEPTUS BEERSI*, N. SP.

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The genus *Dileptus* was established in 1841 by Dujardin and at the present time it embraces 18 species. Descriptions and figures of 17 species were published by Kahl (1931); Wang (1940) described one additional species.

During the summer of 1953 I was informed by Mr. A. C. Broad, a graduate student at the Duke University Marine Laboratory, that specimens of *Dileptus* had been found in water samples taken from Mullet Pond, Shackleford Island, N. C. With his help, collections were made and dilepti were found in abundance. From the large size and mode of feeding, I judged the animals to be *D. monilatus*, a species with which I was quite familiar.

The following summer I returned to the Duke Marine Laboratory to begin an investigation of cystment in this species from Mullet Pond. Specimens were collected and cultures were started. Two weeks were spent in attempting to obtain a satisfactory iron hematoxylin preparation according to the procedure which I had previously found suitable for *D. monilatus*, but the results were less than satisfying. Acetocarmine preparations were finally resorted to and the specimens showed the extreme multinuclearity of *D. anser* without a suggestion of the moniliform pattern so typical of *D. monilatus*. This suggested a new species and observations during 1954 and 1955 have proved this to be the case.

* The author wishes to express his sincere appreciation to Dr. C. G. Bookhout, Director, and to Mr. A. C. Broad, Assistant Director of the Duke University Marine Laboratory for their many kindnesses during the summers of 1954 and 1955 while this investigation was in progress.

During this period, thriving cultures were maintained in the laboratory and studies were made on morphology, feeding habits, fission, trichocysts and cystment. In honor of Dr. C. Dale Beers, Professor of Zoology in the University of North Carolina, I have named this species *D. beersi*, n. sp.

Dileptus beersi n. sp.

Length, 450–900 μ; greatest diameter, 60–70 μ; proboscis $\frac{1}{3}$–$\frac{1}{2}$ the total length; caudal process, 35–65 μ; contractile vacuoles situated aborally, forming two rows extending on either side of the midline from tip of proboscis to caudal process; vacuoles rarely occurring as pairs, usually alternately; nuclear apparatus, 200–500 macronuclei, 6–11 micronuclei. Body spindle-shaped; anteriorly differentiated into a long, flexible proboscis which bears rows of trichocysts on its entire oral surface. Trichocyst rows bounded laterally by a row of long cilia which continues posteriorly to cytostome. Cytostome supported by numerous trichites which are 17 μ in length and lie at about a 45° angle to the longitudinal axis of the body. In a relaxed condition, cytostome circular and 19 μ in diameter. Cytopyge lying immediately anterior to caudal process and emptying toward oral surface of body. Ciliation uniform, the ciliary rows spiralling slightly, 21 μ apart and bearing cilia 6 μ in length. Spherical resting cysts formed. Cysts somewhat variable in size, but usually 100–110 μ in diameter and possessing three cyst membranes. Ectocyst colorless, laminated, sticky and of variable thickness; mesocyst tan to brown in color, 2.5 μ in thickness and inelastic; endocyst colorless and always 0.5 μ in thickness. Food: chiefly small rotifers and *Halteria*; occasionally small flagellates, unicellular green algae and zoogleal masses. Water entering animal along with food or by separate intake through the cytostome. Habitat: fresh to slightly brackish water.

HABITAT—Because of its relative isolation and unusual environment, the habitat of *D. beersi* deserves some special consideration. Shackleford Island is situated about 2 miles off the coast of North Carolina opposite the city of Beaufort. The island is 9 miles long and $\frac{1}{3}$–$\frac{1}{2}$ mile wide. Mullet Pond, situated on the western end of the island, is approximately 100 yards in diameter. Its maximum depth is about 4 feet. A number of years ago, the pond was actually a small bay joined to Back Sound by a narrow inlet. The pond now lies 300 yards inland from Back Sound. Its origin and proximity to salt water cause it to be slightly brackish, having a salinity of 3 parts per thousand. This, of course, is variable as a result of rains and other natural influences such as hurricanes. Aquatic plants, algae, protozoa and small fish are abundant in the pond and attest to its productivity. The wild pigs, sheep, cattle, and horses which roam the island drink there and are contributing factors to this fertility. How this small pond became populated with *Nassula*, *Coleps*, *Loxophyllum*, *Loxodes*, *Euplotes*, *Paramecium*, *Dileptus* and numerous other protozoa is somewhat of an enigma. With those species which form cysts, speculations remain in the realm of reality, but with *Paramecium*, a form which most protozoologists consider to be a non-cystformer, speculations become fantastic.

MATERIALS AND METHODS—Thriving cultures of *D. beersi* were maintained for 18 months using the rotifer *Colurus* sp. as the food organism. *Colurus* grows well in 0.5% timothy hay infusion made up in boiling tap water which is then allowed to stand overnight before inoculation. Subcultures were made at weekly

intervals. The old cultures which contained many rotifers and their eggs were inoculated with several dilepti. Cultures of *Dileptus* started in this manner reached maximum growth in a week to ten days. Gradually the cultures declined and most of the animals encysted. More complex media were tried, but the above procedure, because of its simplicity, was the method of choice. *Halteria* occasionally contaminated the media, but these animals were not detrimental. On the contrary, they were taken as food when rotifers were scarce.

Permanent slides of *D. beersi* were made using Schaudinn's fluid both with and without acetic acid, both heated and at room temperature. I could detect no significant difference in the degree of fixation by the above methods, hence Schaudinn's fluid without acid and at room temperature was used in most cases. Albumen fixative was not used. *D. beersi* like *D. anser* is inherently sticky after fixation and generally becomes firmly attached to the slide. The Feulgen reaction, Heidenhain's iron hematoxylin, Hansen's trioxyhematin, Mallory's triple stain, Grenacher's alum carmine, acetocarmine and aceto-orcein were used to make nuclear studies. Grenacher's alum carmine was the best nuclear stain. A 1.0% aqueous solution of Biebrich scarlet was used to stain trichocysts. Under the phase contrast microscope this stain differentiated the trichocysts exceptionally well.

No method has been found to narcotize *D. beersi*. Like *D. anser*, the animal is constantly in motion, and viscous media like aqueous methyl cellulose produce cytolysis rather than inhibition of movement. Specimens were immobilized in the living condition by means of a makeshift compressorium. This was made by supporting a coverslip sufficiently to squeeze, but not crush the animals beneath it. A vaseline seal was made to prevent evaporation; thus oil-immersion observations could be made on the entrapped animals for several hours before they died.

OBSERVATIONS—*Dileptus beersi* is generally a bottom-dwelling species. In cultures, the animals may be seen moving slowly about the bottom of the bowl probing for food. When present, masses of zooglea, algae, water mold or sand appear to be favorite feeding places and great numbers of dilepti tend to collect and feed on the rotifers and small ciliates which creep about these aggregations. This species feeds with deliberation, often probing rotifers with its proboscis three or four times before attempting to eat them. *D. beersi* does not form a caudal thread (Jones, 1951; Canella, 1951), and hence does not attach itself to objects like *D. dimorphus* (Wang, 1940) or *D. anser* (Jones, 1951).

In old cultures, *D. beersi* forms resting cysts readily. When sand grains or bits of hay are available, the cysts are almost always cemented to them. It is not unusual to find 3–5 cysts gluing several sand grains to the bottom of the dish. Figure 1 shows a typical cyst. In the resting condition, the cysts are spherical or nearly so, rarely less than 100 μ in diameter and the two outer cyst membranes may be clearly distinguished. The outermost layer, the ectocyst (EC) is laminated, colorless or pale yellow and sticky. It may be of uniform thickness or may vary considerably from one side of the cyst to the other. The ectocyst is 3–16 μ in thickness. The mesocyst (MC) is intimately in contact with the inner surface

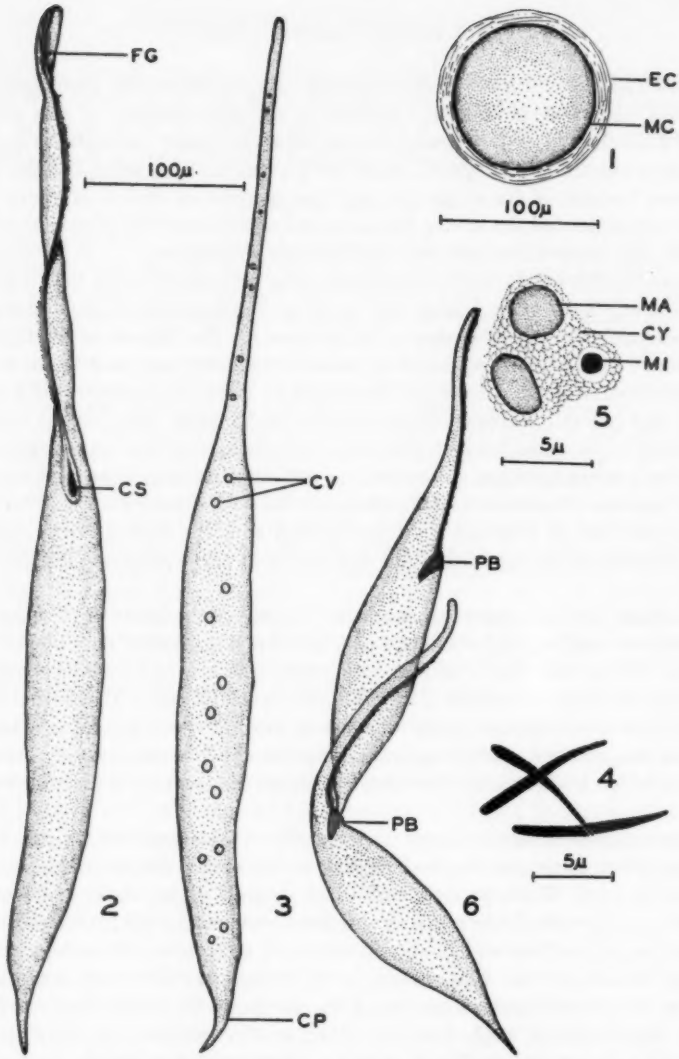

FIG. 1–6. The holotrichous ciliate *Dileptus beersi*, n. sp. FIG. 1. Resting cyst, showing the two outer cyst membranes; endocyst cannot be seen. Drawn from photograph. FIG. 2. Whole mount of mature animal, oral aspect showing the long cilia of the feeding groove. Body ciliation has been omitted. Drawn from photograph. Schaudinn-alum carmine. FIG. 3. Whole mount of mature animal, aboral aspect showing contractile vacuole alignment. Drawn from photograph. Schaudinn-alum carmine. FIG. 4. Unexploded trichocysts from smear. Schaudinn-Biebrich scarlet. Drawn from phase contrast photograph. FIG. 5. Nuclear types, two macronuclei and one micronucleus. Schaudinn-iron hematoxylin. Drawn from photograph. FIG. 6. Sketch drawn from life showing an animal in late division just prior to separation. CS, cytostome; CP, caudal process; CV, contractile vacuoles; CY, cytoplasm; EC, ectocyst; FG, feeding groove; MA, macronucleus; MC, mesocyst; MI, micronucleus; PB, pharyngeal basket.

of the ectocyst. In the young cyst it is yellowish, but in a few days assumes a dark brown color which it never loses. This membrane is always 2.5 μ in thickness and appears to be uniform, lacking an emergence pore. It is relatively brittle and becomes cracked about its circumference during excystment. The endocyst, though present and demonstrable by special technics, cannot normally be seen in the resting cyst. Hence, it is not shown in Fig. 1.

Figure 2 shows a trophic specimen in oral (ventral) aspect. A feeding groove (FG) extends from the tip of the proboscis posteriorly, terminating at the cytostome (CS), a slightly elevated structure supported by numerous trichites which are difficult to see in the living condition, but may be easily demonstrated by staining with Gram's or Lugol's iodine. Hundreds of trichocysts are set perpendicularly to the feeding groove and extend along its entire length. Unexploded trichocysts are blade-like structures 10 μ long and 0.6 μ wide (Fig. 4). They not only occur in the feeding grove, but may be seen moving about in the cytoplasm of the proboscis and body. In stained preparations, protrichocysts have also been identified in the cytoplasm. The fully exploded trichocyst is 40 μ long and is composed of a shaft 12 μ in length which terminates in a 28 μ thread. There can be little doubt as to the function of trichocysts in *D. beersi*. These structures which arm the proboscis make it a formidable food-capturing weapon. The feeding groove is bounded on either side by a row of powerful cilia which sweep the stunned or killed food organisms toward the mouth.

Figure 3 shows an aboral (dorsal) view of *D. beersi*. From this aspect the contractile vacuoles (CV) are most clearly observed. The vacuoles begin at the tip of the proboscis and continue in two rows lateral to the midline as far as the caudal process (CP). Vacuoles situated in the proboscis are always smaller than those found elsewhere in the animal. In transitional forms, *i.e.* excysting or encysting animals, the vacuoles are not aligned. Each vacuole has its own emptying pore, a space in the ectoplasm which lacks cilia and interrupts the trichocystosome-kinetodesma network.

TABLE I

Comparison of Dileptus anser and Dileptus beersi

	D. anser	*D. beersi*
Total length	250–600 μ	450–900 μ
Proboscis length	⅓–½ total	⅓–½ total
Caudal thread	present	absent
Contractile vacuoles	single dorsal row	two dorsal rows
Macronuclear number	100 plus	200–500
Macronuclear size	4.5 μ	3 μ
Macronuclear division	simultaneous	independent
Micronuclear number	16 plus	6–11
Micronuclear size	1.8 μ	1.2 μ
Micronuclear division	rapid	rapid
Cyst membranes	3	3
Cyst size	80 μ	100–110 μ

Typical macro- (MA) and micronuclei (MI) are shown in Fig. 5. Using a smear technic with acetocarmine, nuclear counts were made on 25 animals. Numbers of macronuclei ranged from 203–479 per animal. Micronuclear counts ranged from 6–11. Unlike the macronuclei of *D. anser*, these nuclei do not contain karyosomes (endosomes). In every specimen observed, numerous macronuclei were seen in various stages of division. This was equally true of excysting and encysting animals. The macronuclei of *D. beersi* are 3 μ in diameter.

The micronucleus of this species is composed of a Feulgen-positive core 1.2 μ in diameter which lies in a clear area (micronuclear halo) 2 μ in diameter. In general, the structure is identical to that of the micronucleus of *D. anser*, but each is smaller. Micronuclear division has not been seen, though early post-division stages have been identified. Probably micronuclear divisions are rapid as in the case of *D. anser*.

Table I compares *D. beersi* with *D. anser*, the type species of this genus. *Dileptus anser* is probably the most commonly encountered species and for this reason it was selected for the comparison.

Unlike *D. anser*, cytokinesis in *D. beersi* is completely independent of karyokinesis. The beginning of fission is first indicated by a slight elevation about midway on the aboral surface. This small knob is the primordium of the proboscis of the posterior daughter. The dividing animal swims about slowly as differentiation takes place. By the time that the proboscis of the posterior daughter has differentiated, the anterior daughter is joined only by its caudal process to the cytostomal area of the posterior individual. Figure 6 is a sketch drawn from life of two daughter dilepti just prior to separation.

SUMMARY

A description of the holotrichous ciliate *Dileptus beersi*, n. sp., is given. This species is similar to *D. anser*, but differs from it in over-all size, nuclear number and size, arrangement of contractile vacuoles, and cyst dimensions. Two rows of contractile vacuoles extend from the tip of the proboscis to the caudal process along the dorsal surface and lateral to the midline of the body. *D. beersi* is 450–900 μ in length with a proboscis ½–⅓ of the total length. It possesses 200–500 macronuclei and 6–11 micronuclei. It forms resting cysts, which are 100–110 μ in diameter, and have three cyst membranes. *D. beersi* occurs in fresh to brackish water and is euryphagous, feeding on rotifers, small ciliates and unicellular algae.

LITERATURE CITED

CANELLA, M. F. 1951. Osservazioni morfologiche, biologiche e sistematiche su *Paradileptus estensis* sp. n. e su altri Tracheliidae (Holotricha). Annali Univ. Ferrara, N. S. Sez. III: Biologia Animale **1**: 81–170.

JONES, E. E. 1951. Encystment, excystment, and the nuclear cycle in the ciliate *Dileptus anser*. Jour. Elisha Mitchell Sci. Soc. **67**: 205–217.

KAHL, A. 1931. Die Tierwelt Deutschlands. Urtiere oder Protozoa. I: Wimpertiere oder Ciliata. 21. Teil. Jena: Verlag von Gustav Fischer.

WANG, CHIA-CHI. 1940. Notes on some freshwater infusoria. Sinensia **11**: 11–32.

STUDIES ON MEIOSIS IN CHLAMYDOMONAS[1]

BY MOSELIO SCHAECHTER[2] AND EDWARD D. DELAMATER[3]

INTRODUCTION.—The extensive investigations of Moewus on the genetics of several species of *Chlamydomonas* (see Moewus, 1940, 1951; Sonneborn, 1951, for detailed bibliography) have stimulated other studies on organisms of this genus (notably, Smith and Regnery, 1950; Lewin, 1952a, 1953; Sager, 1954). The studies of Lewin have dealt largely with *C. moewusii*, and have made it possible to consider this organism as a useful genetic tool. Knowledge of meiosis which is desirable or even essential in organisms used in genetic studies, is not available for any species of this genus. Moewus (1936) has presented inconclusive drawings of a few stages of this process in *C. eugametos*, a species closely related to the one used in this study.

C. moewusii has been used exclusively for the initial study of meiosis reported here.

MATERIALS AND METHODS.—The methods of cultivation, microscopy and photomicrography of *Chlamydomonas* have been described previously (Schaechter and DeLamater, 1955). The staining procedure, however, was different, and a full account is given elsewhere (DeLamater, Schaechter and Hunter, 1954). This procedure involves the use of a chemically specific double stain by which nuclei of vegetative cells and of zygotes stain blue and the primary zygote membranes (Lewin, 1952b), magenta. This affords excellent differentiation of these two types of cells which cannot always be easily identified in preparations made with specific nuclear stains. The method, in brief, is as follows:

Impression smears are made on coverslips, and fixed, before drying of the cells, by 5-minute immersion in Carnoy's solution (25 per cent acetic acid in absolute ethanol). They are then subjected to hydrolysis in 1 N HCl at 60°C. for 5 to 8 minutes, and are subsequently stained in 0.2 per cent aqueous azure A for 2 to 4 hours. After thorough rinsing in distilled water, they are treated with 2 per cent thionyl chloride for 2 minutes, and are stained in 0.1 per cent basic fuchsin for 1 minute, or in Schiff's reagent for 5 minutes. This is followed by a second treatment with 2 per cent thionyl chloride for 2 minutes. The preparations are then dehydrated according to the procedure of DeLamater (1951), and mounted in a resinous medium.

The procedure for obtaining and handling the zygotes is as outlined below:

a. *Mating.*—Agar cultures of the two mating types (4 to 6 days old) are suspended in distilled water and mixed. They are illuminated with fluorescent light for 1 to 2 hours, and poured on the surface of agar plates containing Beijerinck's medium. The plates are then placed in darkness until all the water is absorbed.

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[2] George Leib Harrison Fellow, 1953–55.

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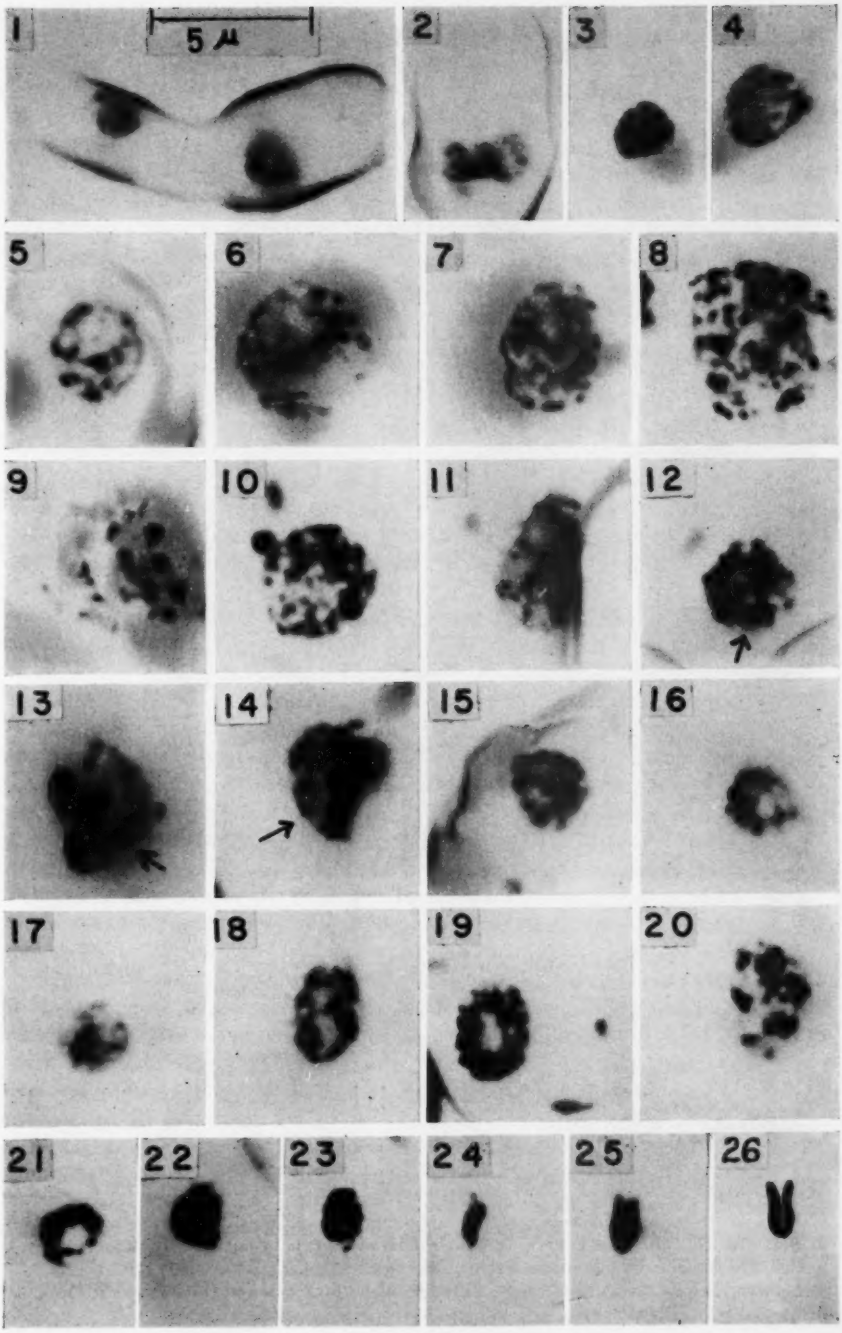
b. *Germination of zygotes.*—Germination is brought about by adapting the procedure of Lewin (1949), which consists of illuminating the plates containing the zygotes for 24 hours, and subsequently placing them in darkness until germination takes place. In the present work, however, no germination occurred if the initial illumination was carried out for less than 40 or more than 50 hours. Since, of necessity, this was done at room temperature rather than at the 23°C used by Lewin, the difference in these two values of initial illumination may be due to moderate fluctuations in temperature in the present experiments.

The zygotes are placed in darkness in order to avoid the formation of a characteristic thick wall which appears about these cells after two or three days of illumination. These walls do not allow the penetration of dyes in the technique here employed. Moreover, if the formation of such structures is allowed to proceed beyond a critical point, germination of the zygotes is inhibited.

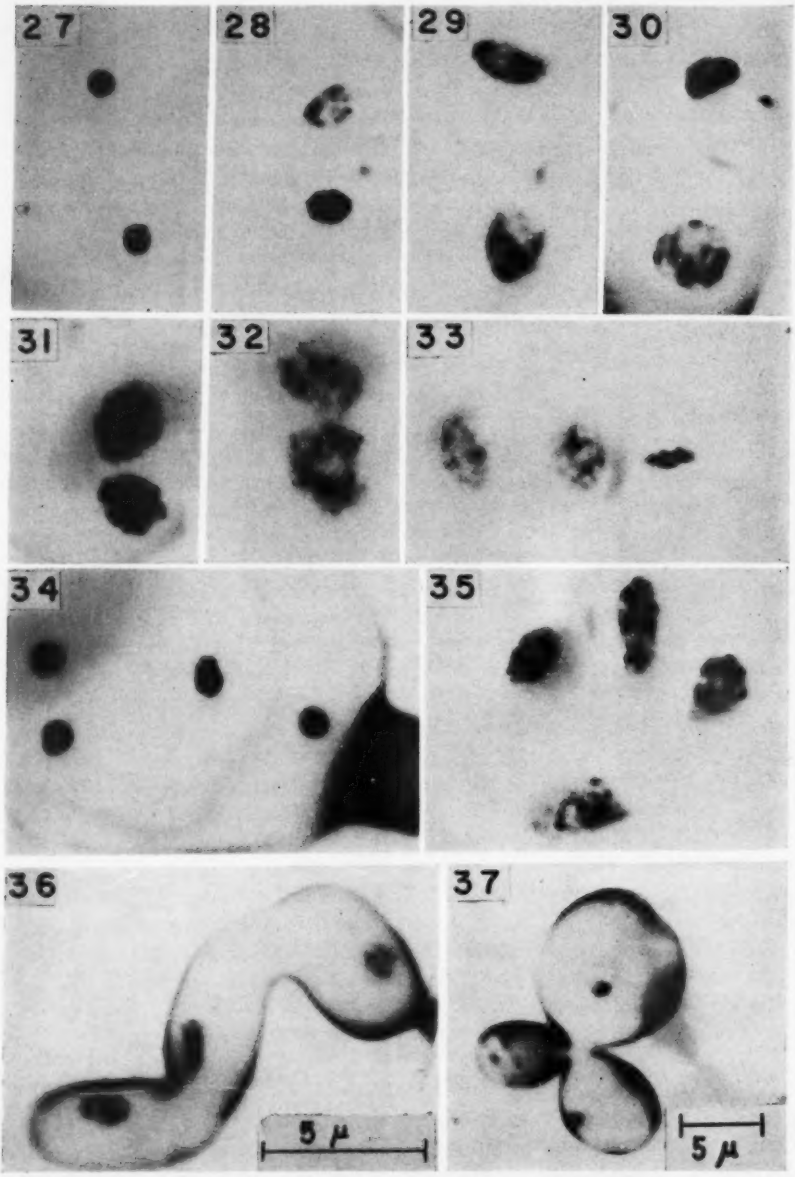
OBSERVATIONS AND DISCUSSION.—The division of the zygote nucleus starts three to six days after zygotes are placed in the dark. In spite of repeated efforts by the investigator, none of the batches of zygotes used in this work showed synchrony in the initiation of germination in all the zygotes present in the preparations. Therefore, a strict time sequence study of the events occurring was not possible. The sequence presented in this article was reconstructed from approximately fifteen trials in the following manner. At the onset of germination a few zygotes show nuclear differentiation. This was interpreted as the early morphological manifestation of germination. After successive time increments new morphological entities were seen in some cells, whereas others showed the previously observed configurations. By following this mode of observation until the end of cell division, all the configurations which newly appeared as the time increased, were considered to be further stages. Nuclear artifacts caused by the death of zygotes were observed in batches which did not germinate at all (having been subjected to improper periods of illumination), and were clearly different from divisional stages. This form of interpretation is not faultless insofar as small morphological changes are concerned; however, the over-all pattern was found consistently throughout this investigation.

Conjugation in *C. moewusii* starts with the entanglement of the flagella of the isogametes, followed by the formation of an intercytoplasmic bridge (Fig. 1). Through this structure the fusion of the two cells takes place. Details of this process have been documented and described by Lewin and Meinhart (1953).

FIGS. 1–26. *Chlamydomonas moewusii*. Magnification 4500×. FIG. 1. Cytoplasmic fusion of isogametes. FIG. 2. Nuclear fusion. FIG. 3. Resting zygote nucleus shortly before onset of meiosis. FIG. 4. Beginning of meiosis. Breakdown of chromatin. FIG. 5. Further breakdown of chromatin into rods. FIG. 6. Filamentation of chromatin. FIG. 7. Chromatinic reticulum. FIG. 8. Paired arrangement of free granules. FIG. 9. Squashed preparation showing paired arrangement. FIG. 10. Squashed preparation to show double filament. FIG. 11. First stage in development of double filament. FIG. 12–16. Progressive stages in development of double filament. FIG. 17–19. Diplotene-like looping. FIG. 20. Diakinesis-like configurations. FIG. 21. Condensation of nucleus. FIG. 22. Further condensation of nucleus. FIG. 23. Metaphase I plate, polar view. FIG. 24. Metaphase I plate, lateral view. FIG. 25. Anaphase I. FIG. 26. Interpretive drawing of FIG. 25.



FIGS. 1-26



FIGS. 27–37. *Chlamydomonas moewusii*. Magnification of Figs. 27–36, 4500×; FIG. 37, 2250×. FIG. 27. Telophase I. FIGS. 28–31. Gradual distension of nuclei. FIG. 32. Interphase nuclei. FIG. 33. Non-synchronous division. Metaphase II and post-division nuclei. FIG. 34. Telophase II. FIG. 35. Zoospore nuclei. FIG. 36. Abnormal fusion of three zygotes. FIG. 37. Abnormal tripolar fusion.

Within 24 hours after mating, fusion of the two nuclei takes place. The two parental nuclei are similar in size and shape to those of vegetative cells in interphase; that is, they are ovoid and show a lightly stainable chromatin reticulum. These nuclei gradually approach one another and fuse by a process that does not reveal any chromatinic differentiation. The fusion can be described as the "flowing together" of the nuclear contents (Fig. 2). The resulting diploid nucleus is elongated, but soon assumes an ovoid shape approximately twice as large as that of the parental nuclei. Toward the beginning of the nuclear division the resting diploid nucleus becomes more chromatic and shows heavily stained granules (Fig. 3).

Three to six days after the zygotes are placed in the dark, they distend and become spheroidal, with two polar protuberances which stain more heavily than the remainder of the cell envelope, and which gradually disappear by further distension of the cell.

The events which take place in the nucleus during the germination of zygotes are described below, and documented in the accompanying photomicrographs. Since the configurations seen in the prophase of the first division cannot easily be considered as parallel to those seen in the classic pattern of meiosis, a purely descriptive terminology will be employed, and the analogies and discrepancies with such a pattern will be considered. Figures 4–7 depict the early nuclear events during zygote germination. They show a gradual filamentation of the chromatin with formation of a resulting fine meshwork in which densely stained granules are seen. This leptotene-like stage is followed by the apparent individualization of the granules as the connecting filaments disappear (Fig. 8, 9). Certain granules are paired. This stage could be considered to be zygotene if it were not for the fact that the number of granules which can be seen is much lower than the 2n number of chromosomes expected (the haploid number of chromosomes in this species has been shown to be 36 ± 2, Schaechter and DeLamater, 1955). Another irregular feature, which is found through the first prophase, is that the granules seen are usually smaller than mitotic chromosomes. Therefore, it is not possible at the present time to determine if these granules represent individual chromosomes. As further stages (Fig. 10–16) develop, there is formation of an annular configuration, which occurs by the elongation of a double filament of chromatin at the periphery of the nucleus. The intimate details of the behavior of the chromatin in these stages could not be studied definitively because of the proximity of the structures. A pair of granules more strongly stained than the rest is seen in Fig. 11–14. It seems likely that the annular shape of the nucleus is due to a central nucleolus.

Configurations which are suggestive of diplotene are seen in Fig. 17–19, where approximately ten loops of chromatin are seen. The photograph reproduced in Fig. 20 shows a figure which was seen in one instance only, showing about ten chromatinic elements in cross-bar configurations, such as those which are seen in diakinesis. At the termination of the first prophase the nuclear ring condenses and contracts (Fig. 21, 22) until a metaphase plate appears (Fig. 23, 24), which is small in size, strongly stained, and substantially flat and disc-like. The small

structure protruding from the nuclei in Fig. 22–24 cannot be interpreted with certainty.

The metaphase plate divides equatorially (Fig. 25, 26) to give two daughter plates, which are also condensed and remain in this condition until completely separated (Fig. 27). The daughter nuclei expand (usually nonsynchronously) into chromatinic reticula resembling interphase nuclei of vegetative cells (Fig. 28–31).

The second division usually occurs non-synchronously. The reticular chromatin of the prophase stage (Fig. 32) condenses into a metaphase plate (Fig. 33) which divides, giving daughter nuclei as dense as those found in the first telophase (Fig. 34). By distension, four daughter nuclei are obtained which acquire the morphological characters of interphase nuclei (Fig. 35). The formation of the zoospores is completed with the deposition of the cytoplasm and cell wall about each nucleus.

If the zoospores are illuminated in the presence of water, they become motile within a short period of time after liberation from the maternal wall. Occasionally zygotes give rise to eight zoospores by an additional mitotic division. Unlike Lewin's observations (1949), this was found to be independent of the time of initial illumination of the zygotes.

During the course of this investigation only two instances of simultaneous cytoplasmic fusion of three gametes were observed. In one case (Fig. 37) the gametes fused at the same pole. The other case (Fig. 36) shows fusion at the opposite poles in two cells, and in normal fashion in the other conjugation. Nuclear fusion in such zygotes was not observed.

CONCLUSIONS.—The nuclear configurations observed in this investigation of the germination of zygotes of *C. moewusii* correspond to those of a meiotic process, in view of the fact that they are seen in the division of a sexually-formed cell which gives rise to zoospores. The details of the process with regard to chromosome behavior are very difficult both to observe and to interpret. This is due to the small size and closeness of the structures observed. Unlike what is seen in classic meiosis, the meiotic nucleus is not very much larger than the mitotic nucleus. Unexpectedly, the elements which could be considered to be chromosomes in meiosis are not larger, but in many cases smaller than the mitotic chromosomes of this organism.

During several stages of the first prophase the number of granules, thought to be meiotic chromosomes, is in the order of magnitude of 10 instead of either the haploid or the diploid number. This allows the possibility that the mechanism of meiosis in this organism is of some aberrant form.

In spite of the interpretative difficulties, it is felt that the sequence of events presented here is a true representation of the meiotic process in this organism, since (a) the study was carried out with a technique which produces a minimum of artifacts, as demonstrated in previous studies on a great variety of cytological material (DeLamater, 1953); (b) although the sequence of events presented is a reconstruction necessitated by our inability to obtain synchronously dividing cultures, it was repeatedly observed in different experimental series. It appears

to be the most likely of the possible alternative explanations which were considered.

As far as we are aware, no detailed descriptions of meiosis in unicellular Volvocales have been published. The smallness and compactness of the nucleus during this process in *C. moewusii* may also be found in other organisms in this group.

SUMMARY

The morphology of the meiotic nucleus of *Chlamydomonas moewusii* was studied with the use of a chemically specific double stain. In this procedure the primary zygote membrane stains magenta; the nuclei stain blue.

A sequence of the morphological events has been reconstructed from the study of batches of zygotes which were not dividing concomitantly. The classic chromosome configurations were not seen during the first division. This was due to the small size and compact arrangement of the structures, which did not permit unequivocal identification of individual chromosomes. It is necessary to conclude that meiotic chromosomes in this organism are remarkably smaller than those found in mitosis.

During the first meiotic metaphase a highly chromatic plate is seen. The daughter nuclei remain compact and strongly stainable until they reach opposite poles of the cell. A characteristic interphase is seen between the first and the second divisions. During the prophase of the second division the chromosomes are not strongly condensed and remain filamentous.

Metaphase, anaphase and telophase of the second division resemble the corresponding stages in the first division. Four zoospores are usually formed under the conditions here employed.

LITERATURE CITED

DELAMATER, E. D. 1951. A staining and dehydrating procedure for the handling of microorganisms. Stain Tech. **26:** 199–204.

DELAMATER, E. D. 1953. Structure and Division of the Bacterial Nucleus. Symposium on Bacterial Cytology, VI International Congress for Microbiology, Rome, 1953. Rend. Istituto Super. di Sanita, 108–135.

———, M. SCHAECHTER AND M. E. HUNTER. 1955. A chemically specific double staining technique for desoxyribosenucleic acid and other aldehyde-yielding or aldehyde-containing substances. Jour. Histochem. and Cytochem. **3:** 16–21.

LEWIN, R. A. 1949. Germination of zygospores in *Chlamydomonas*. Nature **164:** 543–544.

———. 1952a. Ultraviolet induced mutations in *Chlamydomonas moewusii* Gerloff. Jour. Gen. Microb. **6:** 233–248.

———. 1952b. The primary zygote membrane in *Chlamydomonas moewusii*. Jour. Gen. Microb. **6:** 249–250.

———. 1953. The genetics of *Chlamydomonas moewusii* Gerloff. Jour. Genetics **51:** 543–560.

——, AND J. O. MEINHART. 1953. Studies on the flagella of algae. III. Electron micrographs of *Chlamydomonas moewusii*. Canad. Jour. Bot. **31:** 711–717.

MOEWUS, F. 1936. Faktorenaustausch insbesondere der Realisatoren bei *Chlamydomonas* Kreuzungen. Ber. d. Deutsch. Botan. Ges. **54:** 45–57.

———. 1940. Die Analyse von 42 erblichen Eigenschaften der *Chlamydomonas eugametos* Gruppe. Z. Indukt. Abstamm. u. Vererb. Lehre. **78:** 418-522.

———. 1951. Die Sexualstoffe von *Chlamydomonas eugametos*. Ergebn. Enzymforsch. **12**: 173–206.

SAGER, R. 1954. Mendelian and non-mendelian inheritance of streptomycin resistance in *Chlamydomonas reinhardi*. Proc. Nat. Acad. Sci. **40**: 356–363.

SCHAECHTER, M., AND E. D. DELAMATER. 1955. Mitosis of *Chlamydomonas*. Amer. Jour. Bot. **42**: 417–422.

SMITH, G. M., AND D. C. REGNERY. 1950. Inheritance of sexuality in *Chlamydomonas reinhardi*. Proc. Nat. Acad. Sci. **36**: 246–248.

SONNEBORN, T. M. 1951. Some current problems of genetics in the light of investigations on *Chlamydomonas* and paramecium. Cold Spring Harbor Symp. Quant. Biol. **16**: 483–503.

A THEORY ON THE FORMATION OF CYPRESS KNEES

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The spire-like outgrowths from the roots of southern cypress, *Taxodium distichum* (L.) Richard, are familiar to all who have lived or traveled in southeastern North America. They are called knees probably because they always form on a slightly bowed root and, in the early stages, resemble a human knee-cap. As they become older they assume the form of a flattened spire with the long diameter parallel to the root on which they grow. They may become 50 or more years old and reach a height of 5 or 6 feet.

The name pneumatophore, sometimes applied to cypress knees and other similar structures, suggests that these structures promote gas exchange between the often submerged root and the air. Many botany textbooks definitely state this to be the case. Several investigators have made observations or carried out experiments on the formation of cypress knees or on their structure and formation. In a recent paper, Kramer *et al.* (1952) state that "little gas exchange occurs between knees and the root systems on which they occur, and there is no evidence that they play an essential role as aerating structures for the roots." A review of the literature indicates that knees form in a paludal (swamp) habitat only.

Knee formation is an hereditary expression of certain species, since several species may grow in the same habitat and only one produce knees. A clue to the stimulus which brings about knee formation is apparent from a consideration of the microhabitat at their point of origin. The writer has never observed knees forming in water which stands permanently a foot or more in depth—a habitat where they would be most beneficial were they aerating organs. Neither do they form in sites where the soil is well drained although cypress grows well there. Knees do form abundantly, however, at the water's edge of ponds, in swamps, and at stream borders. They are most numerous in swamps along streams where the area is flooded in winter and spring but where the water

level in late spring and summer is at or just below the root level. The writer has lived in the cypress region most of his life and for a number of years worked in swamps where they grow. During these years of almost daily observation, a clear-cut exception to the above statements has never been found. Where apparent exceptions have been found, such as the occurrence of knees on roots in standing water or of knees in well-drained soil, examination of the habitat has shown that water does stand at or near root level during much of the growing season. Many cypress swamps may be flooded with several feet of water during the winter and early spring and in late summer or autumn one may walk dry shod among thousands of knees. It can be seen, however, that there are few if any knees on "islands" nor are there any in pools that remain throughout the year.

The postulate is offered that the stimulus which gives rise to a cypress knee is increased local aeration due to chance emergence of one side of a root just submerged in water or flooded soil. Adequate oxygen allows very rapid growth of the cambium at this point as compared with the remainder of the root. Once a knee begins to grow, its upper end receives, on the average, more oxygen over a longer period of time each year than other parts of the root. Consequently, it continues to grow much faster than the rest of the root even if in standing water most of the time. One can find knees both in standing water and far above the water table, but each began growth when the lower side of the root was submerged and the upper side in air.

Once this theory was formulated, situations which tend to confirm it were found in abundance. In spring, in a swamp where the water table and roots are just at the soil level, hundreds of little knobs can be observed forming on exposed parts of roots. The bursting old cork reveals new cork with the greenish phloem beneath. Sometimes several of these swellings may occur on a single root within a distance of two feet. Long-time observations would be necessary to determine whether most of these become "full fledged" knees or whether continued growth of the knee is in many cases later repressed. As will be pointed out below, careful well-controlled experiments over a period of years, together with extensive gas sampling, would be necessary to give confirmation to the writer's theory. Several field experiments have been undertaken to test the theory but in each case disturbances by man or other animals have terminated the work before definite evidence could be obtained.

Recently, a chance occurrence at a mill pond near Newport, North Carolina, has given support to the validity of the theory. A concrete wall at the spillway of this pond broke several years ago and lowered the water level in the pond about two feet (Fig. 1). This exposed several cypress trees growing in the pond near the spillway to a stream of rapidly flowing water, instead of a much slower current at a higher level. A number of roots had some of the soil washed away so that the younger, distal ends were surrounded by a rapid current while the basal ends were still covered by black muck soil. Within two years a great enlargement of the younger and well-aerated ends occurred as compared with growth of the poorly aerated basal ends. In Fig. 3 are outlines of several of these

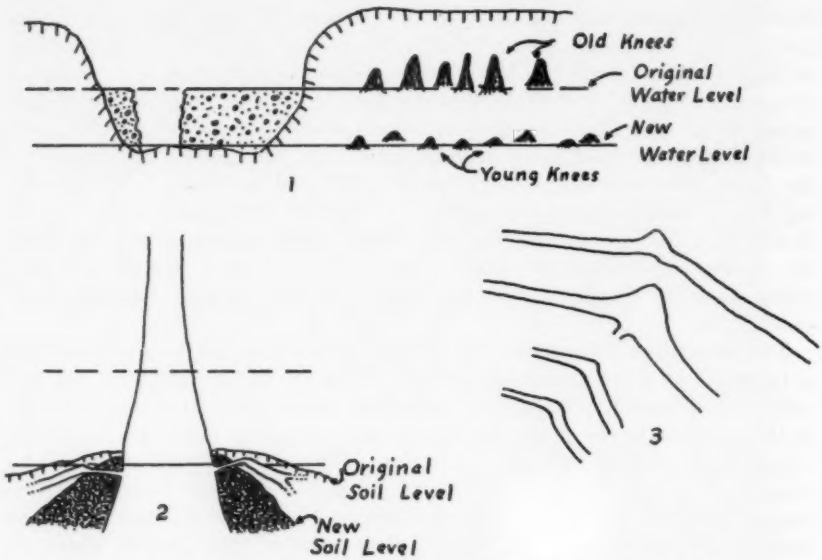

FIG. 1. At left is shown break in concrete wall which lowered water level about two feet. At right is shown older cypress knees which formed at higher water level, and below young knees forming at lower water level. FIG. 2. Sketch of a cypress tree near spillway showing how soil washed away exposing ends of roots to a rapid current but leaving basal portions covered with organic soil. FIG. 3. Outlines of roots from the tree in Fig. 2, traced from photographs. The basal (proximal) ends are at left and the tip (distal) ends toward the right. The cross-sectional area of the younger ends averages four times that of the basal, and older, portions.

roots traced from photographs. A sketch of a typical tree showing changes of water and soil level is shown in Fig. 2. Roots of similar trees in other parts of the pond showed no such enlargement. This seems good evidence for the aeration theory proposed above.

At the edge of the pond another observation gives added support to those above. Seven or eight cypress knees had formed at the exact edge of the pond when at the former (higher) water level. During the two years when the pond was low no more knees formed at this level, but a dozen or more new knees did form at the edge at the new water level (Fig. 1).

The formation of cypress knees seems, therefore, to be a response of the cambium of a root growing in poorly aerated soil or water to chance exposure to the air during the spring or early summer. Their continued growth can be explained on the basis of generally more favorable conditions for respiration in the knee as compared with those in the remainder of the root. It is suggested that investigators in favorable locations in the southeast test the theory by the following methods. (1) Dig out and bend up cypress roots growing in shallow water so that short portions are exposed to the air in spring or summer. (2)

Expose the roots of trees growing in well drained soils and cover several feet with an air-tight but non toxic coating (such as paraffin). Then expose small portions of the coated root to the air by scraping away the covering. (3) Submerge roots of cypress trees growing in well aerated soil in a trough of water probably containing enough organic matter to keep the oxygen tension low. Then bend up short portions of the roots so that one side is exposed to the air. If the theory is valid, the production of cypress knees under experimental conditions should not be very difficult.

REFERENCES

BEAVEN, G. F. AND H. J. OOSTING. 1939. Pocomoke Swamp: a study of a cypress swamp on the Eastern Shore of Maryland. Bull. Torrey Bot. Club **66**: 367–389.

BETTS, H. S. 1945. Baldcypress. American Woods. U. S. Forest Service.

GROOM, PERCY AND S. E. WILSON. 1925. On the pneumatophores of paludal species of *Amoora*, *Carapa* and *Heritiera*. Ann. Bot. **39**: 9–24.

HOFMAN, D. 1927. Vergleichende anatomisch Untersuchungen an rezenten Pneumatophoren von *Taxodium distichum* sowie an fossilen Pneumatophoren aus Parschlug in Steiermark. Berg- und Huttenmannisches Jahrb. **75**: 93–106.

KRAMER, P. J., WALTER S. RILEY AND THOMAS BANNISTER. 1952. Gas exchange of cypress knees. Ecology **33**: 117–121.

KURZ, H. AND D. DEMAREE. 1934. Cypress buttresses and knees in relation to water and air. Ecology **15**: 36–41.

PENFOUND, W. T. 1934. Comparative structure of the wood in the "knees", swollen bases, and normal trunks of the tupelo gum (*Nyssa aquatica* L.). Amer. Jour. Bot. **21**: 623–631.

ROBBINS, W. W. AND T. E. WEIER. 1950. Botany: an Introduction to Plant Science. John Wiley & Sons: New York.

SCHIMPER, A. F. W. 1903. Plant Geography upon a Physiological Basis. English trans. Oxford Univ. Press: London.

WELLS, B. W. 1932. The Natural Gardens of North Carolina. Univ. North Carolina Press: Chapel Hill.

ALBINISTIC VARIEGATION OF TOBACCO LEAVES, A PERICLINAL CHIMERA

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INTRODUCTION.—A commonly-occurring phenomenon among certain species of plants is manifest by foliar and floral variegation and by fruit banding. Pantanelli (1905) early reported foliar variegation of such woody plants as *Acer negundo*, *Agave americana*, *Aucuba japonica*, *Buxus sempervirens*, *Citrus limonum*, *Euonymus europaeus*, *Ficus parcelli*, *Hedera helix*, *Ligustrum vulgare*, *Nerium oleander*, *Sambucus nigra*, and *Ulmus campestris*, and also of several herbaceous species, including *Pelargonium zonale* and *Vinca minor*. He regarded variegation as a "malattia constituzionale."

On several occasions, the writer has noted that large, irregular, creamy-white or yellowish-green patches may occur on the leaves of flue-cured, Burley, and oriental types of tobacco. A similar abnormality of tobacco has been reported from various parts of the world, and has been given such names as albinism, albicatio, albinismus, mottling, bondblad, weissblätterigkeit, gelbsucht, hellfleckigkeit, icterus, panaschierung, panachure, and chlorosis. Whether all of these names apply to the same disorder remains doubtful, since a lack of normal development of chlorophyll and other pigments is known to be induced by a variety of causes.

Since albinism in tobacco has been given only meagre attention previously, the writer, assuming that this disorder is always heritable, initiated a study, during 1953, which shows that this assumption is incorrect. These findings are briefly reported at this time.

EXPERIMENTAL FINDINGS.—The material for this study was a variegated tobacco plant uprooted, during the summer of 1953, from a field near Laurinburg, N. C. It survived when transplanted in the greenhouse although the leaves did not recover. However, axillary shoots or suckers soon began to develop, some of which bore only variegated leaves, as was anticipated, whereas others bore only normally green leaves, an unexpected occurrence. In due time flowers were formed on these suckers, and when the resultant capsules had matured, the suckers were removed and others formed. In turn, they were of two kinds as regards the development of variegated and normal foliage.

The nature of the numerical pattern of heritability of this albinistic variegation was sought by a series of 6 reciprocal cross pollinations, involving flowers on the shoots with variegated foliage, those on the shoots with normally green leaves, and additionally, flowers of a normal plant of another agronomic variety of flue-cured tobacco. Self-pollinations of the 3 parent kinds were also made. Portions of the 9 lots of seeds thus produced were sown and approximately 20 plants of each lot were reared to maturity. Contrary to expectation, the foliage of all plants remained free of variegation, i.e. the leaves of all plants of each lot were normally green. Thereupon, assuming that too few plants may have been reared to disclose albinistic individuals, another sowing was made of each of the 9 lots of seed. An approximately equal number of seedlings of each lot was transplanted and grown to maturity with the result that again none of the plants exhibited variegation. Moreover, seed from self-pollinated flowers of the albinistic shoots, when germinated on moist filter paper, became seedlings with entirely green cotyledons.

DISCUSSION.—On the basis of the foregoing experimental findings it is concluded that the type of variegation under consideration is not to be regarded as Mendelian since it did not prove to be seed transmissible. In seeking to account for the occurrence of such variegation it becomes entirely plausible to assume that the stem tissues of the original disordered plant must have been chimerical and therefore capable of giving rise vegetatively to the two kinds of shoots. Unfortunately stem cuttings, from which plants could have been grown, were not made from the variegated plant. If this had been done, the plants arising as

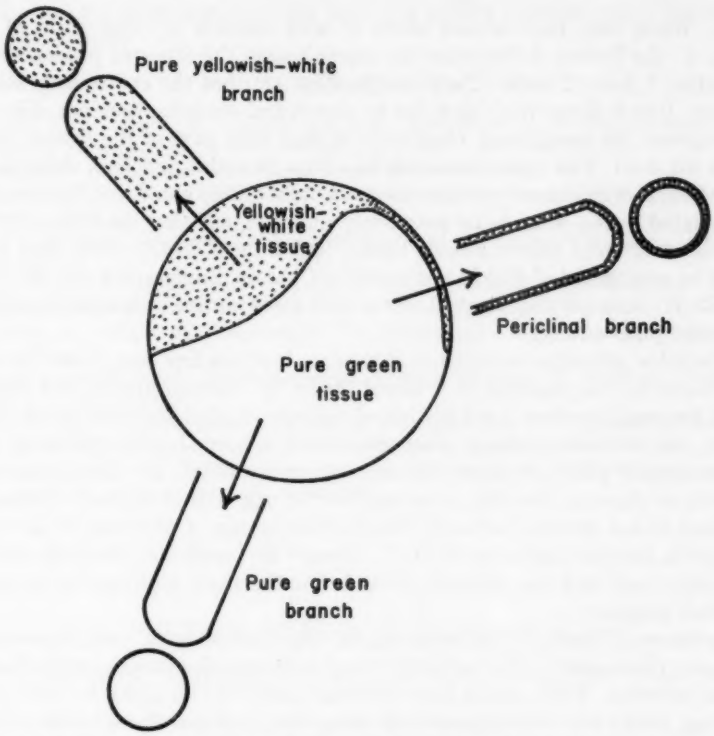

FIG. 1. Transverse section of a chimerical stem, in diagram, to show three potentialities, heritable albinism, heritable normal, and a periclinal albinistic bud sport. The bud primordia occur near the bases of the arrows.

shoots from such cuttings could have been of three kinds, (1) entirely variegated ones, (2) entirely normally green ones, or (3) those constituted of both variegated and normally green tissues, depending upon the localized constitution of somatic tissues comprising the bud primordia (Fig. 1). Such an outcome would have shown conclusively that the abnormality in pigmentation under consideration is a result of bud mutation, or is of a kind commonly called a "bud sport."

The assumption that the variegation in question is probably a bud mutation is supported by the results of experiments by Clausen and Goodspeed (1923). They crossed two varieties of *Nicotiana tabacum*, one parent having carmine flowers and the other, white flowers. Among 200 plants of the F_1 all except one bore only carmine flowers. Certain branches of the inflorescence of this exceptional plant bore only pink flowers and only carmine flowers, on other branches. By means of cuttings from each of the flower branches they raised 2 clones, one of which had only pink flowers and the other only carmine flowers. But all flowers borne on plants arising from root cuttings from the 2 clones were carmine

only. When they back-crossed white ♂ with carmine ♀, and white ♂ with pink ♀, the flowers of the progenies approximated the expected proportion of 1 carmine: 1 pink: 2 white. Their results show (1) that the endogenous somatic tissues, that is those which give rise to carpels and stamens, were not chimerical throughout the exceptional plant but (2) that only part of the dermal tissues were involved. This type of chimera has been termed a periclinal chimera.

Periclinal chimeras are not uncommon. Condit (1928) noted that figs may have variegated leaves, some being completely albinistic, and that the fruits may have bands of different colors. Einset, Blaser, and Imhofe (1947) noted that apples may be constituted of diploid and tetraploid tissues. Zimmerman and Hitchcock (1950–52) observed that single-flowered pink roses formed on a bush of a double-flowered pink variety.

Heritable albinistic variegation of tobacco however has been found to occur, as shown by the findings of a recent study by Dermen (1950). He reported that the seedlings from a self-pollinated variegated plant had light-green cotyledons, and all such seedlings died. Seed from a normal plant pollinated from a variegated plant produced normally green seedlings. He interpreted these results as showing that the cytoplasm of the egg cells is affected whereas the nucleus is not similarly affected. Similar inheritance of albinism of tobacco in Sumatra, has been noted by Dr. P. L. Arens.* He found that albinistic seedlings succumb early and that albinism is masked in the green seedlings but it appears in their progeny.

Instances of heritable chimeras among other species have been demonstrated to occur. For example, Reitz (1940) found a wheat spike having unlike development of awns. From grains from different parts of this spike he grew plants having unlike awn development, showing that germinal tissues were affected. Munerati (1942) observed a beet having sprouts with pure white leaves and other sprouts with pure green leaves. He pollinated flowers of a white-leafed sprout with pollen taken from flowers of the green-leafed sprout with the result that he was able to obtain seedlings some of which had pure white leaves.

Variegated foliage occurred in 58.2% of the seedlings in a population of 216 grown from seed collected from 20 tung trees having variegated foliage (Demaree and Large, 1948).

Different proximate causes have been assigned for the production of chimeras. Some arise "spontaneously," others from the application of certain chemicals, notably colchicine, others from exposure to low temperatures, others from subjection to short-wave irradiation, and others from infection by viruses. Acquaintance can be made with the entire phenomenon of chimeras as regards causes, species involved, and their inheritance by consulting an excellent book by Neilson Jones (1934) to which the interested reader is referred.

SUMMARY

This account deals with a foliar albinistic variegation in flue-cured tobacco. The abnormality was found to be nonheritable. It is concluded to be a chimera

* Personal communication

which probably arose spontaneously and is of a kind included under the term, periclinal chimera.

LITERATURE CITED

CLAUSEN, R. E., AND T. H. GOODSPEED. 1923. Inheritance in *Nicotiana tabacum* III. The occurrence of two natural periclinal chimaeras. Genetics **8:** 97–105.

CONDIT, I. J. 1928. Other fig chimeras. Jour. Hered. **19:** 49–52.

DEMAREE, J. B., AND J. R. LARGE. 1948. Leaf variegation in tung. Phytopath. **38:** 658–660.

DERMEN, H. 1950. The genetics of histogenic type of variegation in tobacco. Genetics **35:** 103–104.

EINSET, J. H., W. BLASER, AND B. IMHOFE. 1947. Chimeral sports of apples. Jour. Hered. **38:** 371–376.

MUNERATI, O. 1942. Die Vererbung der Weissblatterigkeit bei *Beta vulgaris*. Züchter **14:** 214–215.

NEILSON JONES, W. 1934. Plant chimaeras and graft hybrids. VI + 136 pp. Methuen and Co., Ltd.: London.

PANTANELLI, E. 1905. Studii su l'albinismus nel regno vegetale. Malpighia **19:** 45–63.

REITZ, L. P. 1940. An awn chimera in Marquillo wheat. Jour. Hered. **40:** 75–78.

ZIMMERMAN, P. W. AND A. E. HITCHCOCK. 1950/52. Rose "sports" from adventitious buds. Contrib. Boyce Thompson Inst. **16:** 221–224.

NORTH AMERICAN LEJEUNEACEAE

IV. PARADOXAE: COLOLEJEUNEA (CONCL.), DIPLASIOLEJEUNEA

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COLOLEJEUNEA (SPRUCE) SCHIFFN., EMEND. EVS. (CONCLUDED)[2]

SECTIO CALCAREAE sectio nova

Propagulae rotundatae in marginibus, sine cellulis tumidis rhizoido-initialibus (ut in Sectio Minutissimae); folia ovata ad lanceolata, apice acuta vel subacuta, cellulis quasi tuberculos productis.

Leaves with lobes ovate-lanceolate to ovate-pointed, in length *ca.* 1.5–2.0 × width, their margins appearing denticulate (owing to the conoidally protruding cells); sometimes strongly heterophyllous. Lobules often variable, with an apical tooth of 1 or 2 cells, when inflated ovate, *ca.* 0.4–0.5 × the length of lobe; stylus distinct or vestigial. Cells, at least of lobe, tuberculate-produced, the lobes roughened; trigones minute to distinct; oil-bodies delicately papillose, almost homogeneous-appearing; ocelli lacking. Gemmae rarely or not

[1] As in preceding numbers in this series, I wish to express my appreciation for help received in preparation of this paper from my wife, Olga M. Schuster, from Dr. Doris Raymond, from Dr. S. Hattori, and from my colleagues in the Botany Department of Duke University. A portion of the cost of preparation and publication of this paper has been defrayed by National Science Foundation Grant No. 669.

[2] For the initial section dealing with this genus, including keys to species, see Jour. Elisha Mitchell Sci. Soc. **71**(2): 218. 1955.

at all marginal, produced either on antical or on postical faces, rarely on both, similar to those of the Minutissimae, i.e. merely regularly crenulate on margins, without angulate protrusions, and without specialized slime cells (Figs. XII: 9; XIII: 3).

Perianth as in the Minutissimae, but strongly tuberculate, with or without a distinct beak.

The Sectio Calcareae stands midway between the Minutissimae, with which it shares similar gemmae, and the Diaphanae, with which it shares the ability to produce tuberculiform protuberances of the cells (Fig. XII: 5); the ovate to lanceolate, pointed leaf-lobes (Fig. XIII: 1); the frequently marked heterophylly of the shoots, with some lobules much reduced; the faintly papillose, delicately segmented oil-bodies. In addition to the regional *C. biddlecomiae* and *C. contractiloba*, *C. ornata* probably belongs to this complex, exhibiting a close affinity with *C. biddlecomiae* and the European *C. calcarea*.

Cololejeunea contractiloba Evans

Cololejeunea contractiloba Evans, Amer. Jour. Bot. **5**: 131, f. 1, 1918; Evans, The Bryol· **41**: 80, 1938; Frye and Clark, Univ. Wash. Publ. Biol. **6**: 877, 1947; Schuster and Hattori, Jour. Hattori Bot. Lab., No. 11: 64, pl. 14: 3–5, 1954.

Plants extremely delicate, green to pale yellowish green, occurring as scattered stems or in very diffuse, inconspicuous small patches; shoots prostrate, 280–425 μ, rarely up to 475 μ wide, irregular and usually sparsely branched, the branches like main stems, widely spreading. Stems delicate, geniculate-flexuous and sinuate, 30–38 μ in diam., 3–6 mm. long, sparsely rhizoidous. Leaves distant to contiguous, rarely subimbricate, suberect to rather widely spreading (40–60°), the dorsal bases not completely extending across stem. Dorsal lobes slightly convex or plane, the tips often deflexed, ovate-pointed to ovate-lanceolate, (170) 185–190, up to 200–300 μ long × 105–125, up to 150–180 μ wide, gradually narrowed to an *acute, pointed apex*, terminated by a single cell; both dorsal and ventral margins convexly arched on lower half of lobe and virtually straight on distal half, sharply crenulate to denticulate marginally, owing to the strongly conically tuberculate cells. *Lobules heteromorphic*, occasionally to frequently vestigial (reduced to a 2-celled basal fold); when well-developed, inflated, ovate to broadly oblong, *0.45–0.6 × length of lobes*, *ca.* 110–130 μ long × 85–110 μ wide, with keel moderately to strongly arched, *the apical tooth 1-celled, quite inconspicuous, rounded or triangular, projecting ventrally and not lying in the plane of the lobule* (Fig. XII: 2a); hyaline papilla near or at ental base of apical tooth; proximal tooth rounded or obtuse, *larger than apical tooth* but not sharply defined, separated from apical tooth by a single cell, the sinus shallow and short; *stylus vestigial, reduced to an ephemeral hyaline papilla. Cells of lobe dorsally everywhere extremely strongly conically tuberculate*, the dorsal surface of the lobe roughened with large, conical protuberances, one per cell, which are thin-walled except for their thickened apices; keel similarly, often even more strongly armed, consequently sharply denticulate in profile; *cells of face of lobule plane;* marginal cells of lobe 12–14 μ, median cells *ca.* (11) 12–16 × (12) 16–18 μ, thin-walled except for vestigial to discrete, but concave-sided trigones and the thickened tips of the conical protuberances; cells of lobule *ca.* 11–16 × 15–21 μ; oil-bodies 3–5, rarely 5–7 per cell, small, largely fusiform to sublinear or *Paramecium*-shaped (then 2–2.5 (3) × 3–5.5 (6.5–9) μ), but sometimes shorter and spherical to ovoid (then 2.5–3.5 × 3–4 μ), averaging larger than chloroplasts, which are 2.5–3.5 μ, finely segmented and appearing barely papillose, or (rarely) divided into coarser segments (lying in 1–2 rows in superficial view). Asexual reproduction by abundantly produced gemmae, produced from antical and postical surfaces of lobes or occasionally from marginal cells, each *ca.* 50 × 60 μ, broadly orbicular, *normally formed of 18–20 cells* (each apical quadrant cutting off 3 segments), margin crenulate from projecting cells, *lacking organs of attachment*.

Autoecious. Female inflorescence typically on a more or less abbreviated branch that innovates on one side; the innovation typically sterile; bracts rather widely spreading at maturity of perianth, somewhat complicate, the lobe ovate to ovate-lanceolate, like leaf, up to 350–400 μ long × 120–200 μ wide, the apex, margins and surface as in leaves; lobule rectangular to ovate to obovate, to 150–200 μ long × 100 μ wide, rounded to subtruncate or somewhat pointed at apex. Perianth obovoid, to 350 μ long × 250 μ wide, the distal one-half 5-carinate, the keels bluntly angulate below, sharper above; keels and upper portion of perianth-surface roughened by conically tuberculate cells (as on leaves); apex of perianth narrowly truncate or rounded, shortly beaked. Androecia 220–250 μ wide, terminal on abbreviated branches (with 1–3 more or less reduced leaves at base), not proliferating distally; ♂ bracts in 1–2 pairs, like leaves but more suberect, with slightly reduced lobes (*ca.* 140–180 μ long) but somewhat more ventricose lobules (100–120 μ long); monandrous. Capsule 180 μ in diam.; spores 12–20 μ in narrower diam., minutely verruculose.

TYPE: Sanford, Florida, Sept. 28, 1915, *S. Rapp.*

DISTRIBUTION.—Restricted to the tropical and subtropical portions of Florida, westward along the Gulf Coast to Mississippi. Evidently rare and of scattered distribution. Occurring chiefly in shaded, damp hammock forests, and in Red Maple-Bald Cypress swamp and river-bank forests. The species is particularly frequent in rich Beech-Magnolia forests, along the Mississippi Gulf Coast, (on bark of *Magnolia grandiflora*, *M. acuminata*, *Fagus grandifolia*, *Liriodendron*) where it is associated with such subtropical shrubs as *Illicium floridanum*. The recurrence with the latter is remarkable, the *Cololejeunea* occurring with *Illicium* at 2 out of the 3 Mississippi stations, and with it in the Ocala National Forest and in western Florida. Occasionally also in *Magnolia-Ilex-Quercus nigra* forests, where it may occur on *Liriodendron*. In the swampy *Taxodium-Acer rubrum* forests it occurs on the bark of *Acer rubrum tridens*, *Quercus michauxii*, *Taxodium distichum*. When occurring in hammock forests (as at Highlands Hammock), it may occur on various species, occasionally on *Carya*.

The species also occurs in relatively open hammock forest (along Hillsborough River), at the bases of *Juniperus silicicola* and *Quercus virginiana*, associated with *Cheilolejeunea clausa* and *rigidula*, and *Mastigolejeunea auriculata*. It is very rare in the tropical hammocks in the southern tip of Florida (Ross Hammock, Dade Co.), where it occurs on bark.

Although previously reported only from Sanford, Florida, the species appears to be not infrequent in localized, rich, mesic forests.

Georgia: CHARLTON Co. (Okefenokee Swamp, *Blomquist 11509a!*, a trace only, but quite typical). **Mississippi:** JACKSON Co. (near Pascagoula R., 6–8 mi. n.e. of Van Cleave, *RMS 19230a*, *19230d*); GEORGE Co. (Wyatt Hills, n.e. of Ramsey Springs, *RMS 27681*, *20134*, *27620*, *27723*, *27740*); HARRISON Co. (ca. 12–15 mi. n. of Handsboro, Rte. 57, *RMS 27770* [Plate]). **Florida:** SEMINOLE Co. (Sanford, *Rapp*, type); MARION Co. (Juniper Springs, Ocala National Forest, *RMS 22091*; e. bank Oklawaha R., Rte. 40, 4 mi. e. of Silver Springs, *RMS 22453*, *22457*); HIGHLANDS Co. (Highlands Hammock State Park, w. of Sebring, *RMS 26004b*); HILLSBOROUGH Co. (Hillsborough River State Park, *RMS 33930a*, *p.p.*); DADE Co. (Ross Hammock, n. of Homestead, *RMS 33801*, *p. min. p.*); ALACHUA Co. ("Buzzards Roost" 7 mi. w. of Gainesville, *RMS 33348a*, *p.p.*, over *Dumortiera hirsuta*, with *Cololejeunea ornata*, *Lejeunea calcicola*, *L. minutiloba*; same locality, *RMS 33349a*, *33350*); LIBERTY Co. (Alum Bluff, Appalachicola R., *RMS 33532a*, *p.p.*, with *Lejeunea cladogyna*, *Radula caloosiensis*); MARION Co. (Juniper Creek, 3–4 mi.

below Juniper Springs, Ocala Ntl. Forest, *RMS 33398, 33399, 33396a, p.p., 33465, p.p., 33450, c. capsules*); CLAY CO. (Ravine of Gold Head Branch State Park, *RMS 37203, p.p.*, among *Cololejeunea diaphana cristulata, Aphanolejeunea ephemeroides, Drepanolejeunea bidens sabaliana);* CITRUS CO. (Limestone grottos at Pineola, *RMS 37213a, p.p.*, growing over *Plagiochila ludoviciana*, with *Lejeunea minutiloba*, and *RMS 37224, p.p.*, growing over *Dumortiera hirsuta*, with *Cololejeunea ornata, Lejeunea minutiloba*).

ECOLOGY.—Except for a general limitation to mesic, dense forests, usually to deeply shaded sites, *C. contractiloba* does not exhibit very marked ecological restrictions. The species has been reported only from bark, which is its "normal" habitat, but I have found it creeping over the robust thalli of *Dumortiera hirsuta* growing on vertical coral limestone walls, associated with traces of *Cololejeunea ornata, Lejeunea calcicola* and *L. minutiloba* (*RMS 33348a*).

When on bark, occurring indifferently on the trunks of trees, on their exposed bases or roots, or on dead wood. It almost always grows very close to the ground level, where the humidity is highest, but, unlike the superficially slightly similar *C. setiloba*, does not grow where it is apt to come into contact with standing water. The species is a pioneer, but persists after a variety of other hepatics have undergone ecesis, growing over the more robust of these, much as the closely allied *C. biddlecomiae*. Occurring variously consociated are: *Metzgeria uncigera* and *ciliifera, Plagiochila ludoviciana, hypnoides* and *floridana, Cheilolejeunea rigidula* and *clausa, Ceratolejeunea laete-fusca, Rectolejeunea maxonii, Mastigolejeunea auriculata, Radula caloosiensis, australis* and *obconica*, occasionally *Cololejeunea minutissima* and *Lejeunea laetevirens*. On the lianas of *Vitis* sometimes with *Lejeunea minutiloba* and *Crossotolejeunea bermudiana*; on exposed roots, in very humid areas, with *Lejeunea cladogyna* and *glaucescens, Cololejeunea diaphana, C. minutissima*. In the southern tip of Florida also with *Lejeunea longifissa* and *minutiloba*, and such mosses as *Neckera disticha*, in deeply shaded hammock forests.

DIFFERENTIATION.—Superficially the leaf-shape and very strongly armed lobe-surfaces of this species strongly suggest *C. biddlecomiae*, with which it was for some time confused. It is also related, if more distantly, to *C. ornata*. From both of these species it can be separated most easily by the absence of discrete styli (Fig. XII: 6, 7). *C. ornata* is further differentiated from both *C. contractiloba* and *biddlecomiae* by the fact that the surfaces of the lobules are tuberculate like the surface of the lobes. By contrast, *C. contractiloba* has the cells of the lobules, except adjacent to the keels, smooth and non-tuberculate (Fig. XII: 7). It should be emphasized, however, that occasionally lobules of the very strongly tuberculate extreme of the species (*RMS 27770*) possess isolated, rare cells of the lobular surface, away from the keel, that are provided with low but discrete tuberculate projections. Such cellular tubercles of the lobule are exceptional in occurrence in *C. contractiloba*, regular in occurrence in *C. ornata*. In both species, as well as in *C. biddlecomiae*, the surface of the perianth is quite rough, because of a similar development of conical, cellular tubercles as on the lobes.

The gemmae suggest a further point of difference between *C. contractiloba* and *C. biddlecomiae*. In the former they are formed of *ca.* 18–20 cells and are crenulate

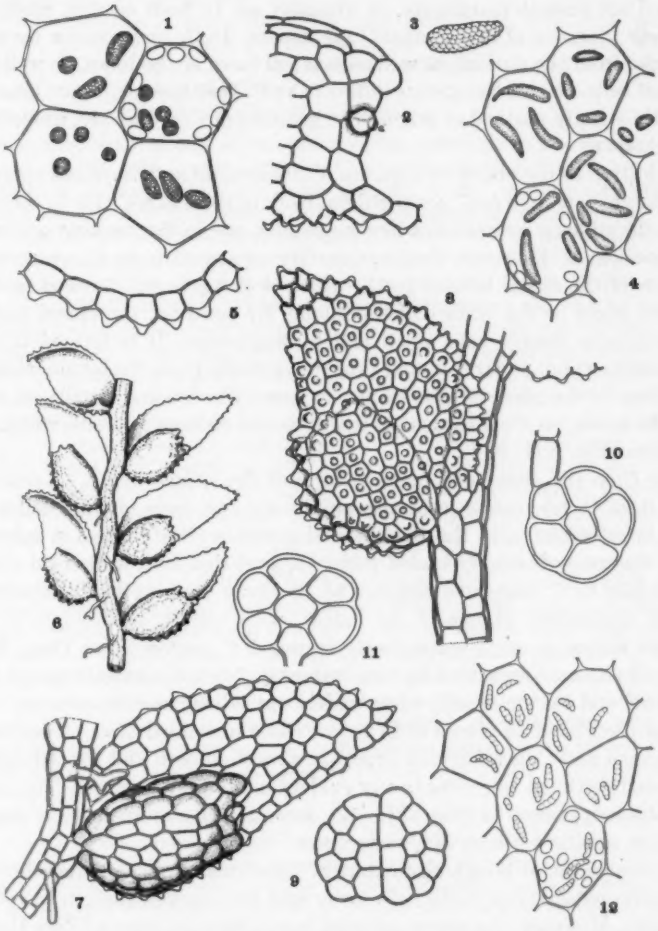

FIG. XII. *Cololejeunea contractiloba* Evans. 1. Median cells (×1100); 2. Apex of lobule, at *a* the hyaline papilla lying virtually hidden beneath the 1-celled apical tooth (×280); 3. Oil-body (×1570); 4. Median cells, the lower cell with chloroplasts drawn in (×845); 5. Cells along keel, optical section (×400); 6. Shoot, postical view (×110); 7. Leaf and adjacent section of stem, showing rhizoids (×285); 8. Leaf, antical view (×247); 9. Gemma (×312); 10–11. Cross-sections through the stem, in *10* with leaf-base indicated, in *11* with rhizoid base (×470); 12. Cells of leaf-middle, of extreme with coarsely-segmented oil-bodies (×665). (Figs. 1, 8. Juniper Springs, Fla., *Schuster;* 2, 10–12, Florida, *Schuster 27770;* 3–4, Florida, *Schuster 26004b;* 5–7, Van Cleave, Miss., *Schuster 19230;* 9, Florida, *Schuster 22453*).

from projecting cells (Fig. XII: 9); in the latter they are formed of *ca.* 26–32 cells and are smooth marginally, or virtually so. In both species, gibbous marginal cells ("organs of attachment") are absent. In *C. contractiloba* the gemmae may arise from the antical, as well as postical faces of the lobes, as well as from marginal cells. In this the species differs from *C. biddlecomiae*, where gemmae are evidently strictly postical in origin. The gemmae of *C. ornata* are, unfortunately, still unknown.

In addition to the above two species, *C. tuberculata* and *diaphana* appear to be more distantly related to *C. contractiloba*. Both of these agree with *C. contractiloba* in that the stylus is reduced to a hyaline papilla, and in the frequent occurrence of elobulate leaves. However, they are readily separated from *C. contractiloba* by the form of the apical lobular tooth, which is elongate and 2-celled, and lies in the same plane as the surface of the lobule. By contrast, the apical tooth of *C. contractiloba* is always very small and inconspicuous. It is formed of a single triangular to subglobular cell, and projects *ventrally* from the lobule, rather than projecting in the plane of the lobule. It bears the hyaline papilla at its ental base; the latter, as a consequence, usually is not distinct in a superficial view of the lobule (Fig. XII: 2, 7).

Aside from the above difference in form of the lobular tooth, *C. contractiloba* differs from *C. tuberculata* in the essentially smooth faces of the lobules (tuberculate in *tuberculata*), in the 18–20-celled gemmae (16–18-celled in *tuberculata*), and in the more distinctly keeled perianth, provided with a discrete beak. The relationship to *C. diaphana* appears to be closer than at first suspected. The recently discovered phases of the latter, with totally tuberculate lobes and narrower leaves, in many ways closely approach *C. contractiloba*. They, however, can at all times be separated by the uniformly 2-celled, elongate apical tooth of the lobule and by the usually more widely spreading, narrower leaves.

All of the Floridian plants of *C. contractiloba* examined have oil-bodies of the *C. diaphana* and *C. biddlecomiae* type, i.e. almost smooth and exceedingly finely segmented, with the spherules barely evident under oil-immersion. The collection from Mississippi figured (Fig. XII: 12), however, has more coarsely segmented oil-bodies, similar to those of *C. subcristata*.

C. contractiloba, although prominantly "tuberculate", usually has the conoidal papilliform outgrowths of the cells lower and less marked than in *C. tuberculata* and *ornata*. However, the sparse material from a deep hammock (Ross Hammock, Florida, *RMS 33801*) has extraordinarily strong development of the "papillae." In this species there is evidently a correlation between an unusually humid habitat, and dense shade, and a maximal development of the "papillae."

Both *C. contractiloba* and *C. diaphana* share the ability to produce phases with almost all of the leaves bearing vestigial lobules. Such forms are frequent in *C. diaphana*, but quite rare in *C. contractiloba* (represented by *RMS 33349a*).

Cololejeunea biddlecomiae (Aust.) Evs.

Lejeunea calcarea Sulliv., Musci Allegh. (Exsic.) No. 275, 1845 (not of Libert, Ann. Gen. Sci. Phys. **6**: 373, 1820).

Lejeunea echinata Austin, Hep. Bor. Amer. (Exsic.) No. 99, 1873 (not of Taylor, in G. L. and N., Syn. Hep. 345, 1845).

Lejeunea biddlecomiae Austin *ex* Pearson, List. Canadian Hep. (Geol. and Nat. Hist. Survey of Canada) 5, pl. 5, 1890.

Cololejeunea biddlecomiae Evans, Mem. Torrey Bot. Club **8**: 168, 1902; Frye and Clark, Univ. Wash. Publ. Biol. **6** (5): 875, figs. 1–12, 1947; Schuster, Amer. Midl. Nat. **49** (2): 557, pl. 69, figs. 4–6, 1953; Schuster and Hattori, Jour. Hattori Bot. Lab. No. 11: pl. 13: 8, pl. 14: 6–8, 1954.

Physocolea biddlecomiae Steph., Sp. Hep. **5**: 912, 1916.

Scattered or in small, closely adnate and creeping, yellow-green patches; shoots prostrate, *mostly 500–700 (750) μ wide*, irregularly and rather freely branched, the branches widely spreading. Stems delicate, somewhat flexuous, weakly geniculate at times, (40) 50–65 μ in diam., 3–9 mm. long usually; subfloral innovation almost constantly present, usually 1; rhizoids 2–4 per locus, short and rigid. Leaves distant to barely imbricate, obliquely to widely spreading (50–65° usually); dorsal lobes ovate-pointed to narrowly ovate, gradually narrowed to the *acute or subacute apex*, *ca.* 320–400 μ long × 200–250 μ wide, in length averaging 2.0–2.4 × length of lobule, usually slightly convex and with the tips somewhat deflexed, *appearing prominently roughened*, the cells each elevated (on abaxial surface) into a salient, median, conical protuberance; margins of lobe more or less serrulate (owing to the more or less marginal position of the conical elevations of the marginal cells). *Lobules usually well developed*, broadly ovate or oval, inflated almost throughout, *ca.* 120–145 μ wide × 170–190 μ long, their *surface cells convex but not conically elevated* (except at keel); free margin of lobule involute to the obtuse and ill-defined preapical tooth, separated rather widely from a *prominent 2(-3-4)-celled apical tooth* lying in the oblique sinus between preapical tooth and keel; keel convex, arched, crenulate at least distally, because of the strongly conically produced cells; *styli prominent, usually uniseriate throughout* (rarely biseriate near base), mostly 5–8, rarely to 10–12 cells long. Cells thin-walled and with minute trigones (except for the thick-walled apex of the conical protuberance), *ca.* 10–13 μ on margins of distal half of lobe, (13–15) 16–18 μ × (15–20) 21–23 μ medially. Oil-bodies appearing *almost homogeneous*, the included globules barely visible (under oil-immersion) and lying in a matrix of nearly equal refractive index, at best obscurely papillose in appearance, from 4–8 to 7–12 per cell, rarely 12–15 per cell, from subspherical and 2.2–2.5 μ to 1.8–2.2 × 4–5 μ up to 2–2.5 × 5–6 μ. Chloroplasts 4–4.5 μ long, subequal to oil-bodies in size or barely larger. Gemmae limited in development to *postical surface of intramarginal cells of lobe*, freely produced, discoid, broadly ellipsoidal, *ca. 26–32-celled when mature* (but of no fixed, determinate size), *ca.* 60–64 × 65–72 μ, the margins nearly smooth or barely and regularly crenulate because of the slightly bulging walls, *without* protuberant slime cells.

Autoecious. Androecia loosely spicate, usually of 3–6 pairs of bracts, *extremely similar to leaves* and essentially unmodified, the lobes acute, ovate or narrowly ovate, the lobules inflated, with usually nearly normal development of the 2-celled apical tooth; styli normally developed; antheridium 1; androecia usually terminal on leading branches, sometimes on short lateral branches. Gynoecia *usually on short lateral shoots*, arising commonly below an androecium, with 1 subfloral innovation. Bracts unequally bilobed, erect-spreading; dorsal lobe broadly ovate, subacute to obtuse or rounded at apex, the margins crenulate owing to elevated cells, to 350 μ long × 200 μ wide; lobule larger than on leaves, united for almost its whole length to lobe, variable, from semicircular to subquadrate, the rounded free margin crenulate owing to bulging cells (and sometimes slightly 2–3-lobed); base of lobule usually with a stylus 1–2 cells wide at base and 5–10 cells long. Perianth somewhat stipitate with age, 500–600 μ long (exclusive of the broad and short beak) × 350–400 μ wide, sharply 5-carinate in distal half, not at all dorsiventrally compressed, rounded or subtruncate distally, exclusive of beak; surface, both on keels and between them, strongly roughened because of the conically projecting cells, except in basal fourth of perianth, which is almost smooth.

TYPE: "On trees, Canada (*Macoun*, Herb. Aust.)" (Pearson, 1890).

DISTRIBUTION.—The most widespread species of *Cololejeunea* in North America, and by far the most abundant. The species is essentially of temperate

and sub-boreal distribution, ranging from the southern half of the Spruce-Fir forest, throughout the Deciduous Forests, into the broad-leaved evergreen forests of the southeastern Coastal Plain. It is replaced in Florida by the allied *C. ornata* and *contractiloba*. *C. biddlecomiae* appears to be the eastern American representative of the old, Tertiary complex formed by the European *C. calcarea* and the American *C. biddlecomiae*. Unlike the rest of our species of the genus, this species has a distribution essentially derived from an Appalachian center.

Canada: QUEBEC: Hull (Lepage, 1945). ONTARIO: "On cedar trees", Dow's Swamp, Ottawa, and Belleville (Macoun, 1902); Carlton and Hastings Cos.; also Muskoka Distr. NOVA SCOTIA: Wobamkek, Glen Margaret; Port Mouton; Lochaber; Cow Bay Run (Brown, 1936). NEW BRUNSWICK: (Evans, 1938).

United States: MAINE: Buckfield. NEW HAMPSHIRE: Cornish; Hanover; Wildwood Path, Franconia Mts. (Lorenz, 1908). VERMONT: Newfane (Grout). MASSACHUSETTS: Leicester. CONNECTICUT: Litchfield, Hartford, Fairfield, New Haven Cos. NEW YORK: Warren Co. (Burnham); Tompkins, Cortland, Onondaga, Schuyler, Madison, Cayuga and Tioga Cos. (Schuster, 1949); Herkimer Co. (Little Moose Lake); Farmington (Underwood and Cook, 1889, as *Lejeunea calcarea*). NEW JERSEY: no loc. (Evans, 1902). PENNSYLVANIA: Center and Bedford Cos.; Sayre (Barbour, 1901; as *L. calcarea*). DISTRICT OF COLUMBIA: Rock Creek (as *L. calcarea;* see Holzinger, 1907). WEST VIRGINIA: Braxton, Campbell, Lincoln, Monongalia, Preston, Ritchie Cos. (Ammons, 1940); Mouth of Seneca, Monongahela Ntl. Forest (*RMS 18302*); w. slope Shenandoah Mt., e. of Brandywine (*RMS 18371, 18381*). VIRGINIA: Botetourt, Charles City, James City, King and Queen, Roanoke, Rockbridge, Spottsylvania, Sussex Cos. (Patterson, 1950); Norfolk Co. (Lake Drummond, Dismal Swamp, *RMS and Patterson*); Nansemond Co. (*ca.* 3–5 mi. n.e. of Cypress Chapel, *RMS and Patterson; Plate*); Giles Co. (Mountain Lake). KENTUCKY: Carter, Harlan, Lewis, Powell, Letcher, Whitley, Wolfe Cos. (Fulford, 1934, 1936); Cumberland Falls, Whitley Co. (*Sharp and Wells KL-541*). TENNESSEE: Knox, Rutherford, Sevier and Washington Cos. (Sharp, 1939); Blount, Morrison, Green Cos. (Morrison, 1938); Montgomery Co. (near Clarksville); Blount Co. (Middle Prong of Little R., 1600 ft., below Tremont, *RMS and A. J. Sharp 34622b*).

NORTH CAROLINA: Durham, Forsyth, Harnett, Mecklenburg, Moore, Orange, Rutherford, Wake, Watauga Cos. (Blomquist, 1936); Swain Co. (Soco Falls near Cherokee, *RMS 24726b, 24621e*); Pender Co. (n.e. Cape Fear R., 10–12 mi. n. of Wilmington, *RMS 33466*); Columbus Co. (1.5 mi. e. of Whiteville, *RMS 30041, 33561*); Wake Co. (Hemlock Bluff near Cary, *RMS 28690a, 28726, 28693a*); Harnett Co. (Raven Rock, near Mamers, Cape Fear R., *RMS 28682*); Dare Co. (Collington, *RMS 28301a*); Durham Co. (Duke Forest, Durham, *RMS 30125*); Stokes Co. (Cascades, near Hanging Rock State Park, *RMS and Blomquist 28233, 28231*); McDowell Co. (6 mi. n. of Marion, *RMS and Jones 28830d, 29384*); Columbus Co. (Lake Waccamaw, *RMS and Blomquist 30021*); Washington Co. (near Roper, *RMS 28354, 28361, 28369c, 28357*); Durham Co. (Eno R., 4 mi. n. of Durham, *RMS and Blomquist 28422*); Ashe Co. (Bluff Mt. near W. Jefferson, *RMS and Jones 30079*); Hyde Co. (near Scranton, *RMS 28774*); Orange Co. (New Hope Creek at the Caves, *RMS and Blomquist 28530*); Beaufort Co. (Pungo Creek, *Blomquist 8139*); McDowell Co. (1 mi. w. of Old Fort, *Blomquist*); Polk Co. (4 mi. n.w. of Tryon, below Melrose Falls, *RMS 36048 p.p.*, among *Lejeunea cavifolia*).

SOUTH CAROLINA: Oconee Co. (Whitewater Falls Gorge, near Jocassee, *RMS*); Bamberg Co. (Swamp 8 mi. s. of Bamberg, *RMS 33977, 33981, 33983b*). GEORGIA: Thomas Co. (Thomasville, *M. S. Brown*); Towns Co. (Brasstown Bald, 4000–4500 ft., *RMS 34320a*). MISSISSIPPI: Tishomingo Co. (Tishomingo State Park, *RMS 19701;* below waterfall, near Pickwick Reservoir, *Schuster 19708*). INDIANA: Parke Co. (Turkey Run State Park, *RMS 18333; Plate*); Montgomery Co. (Wood). MICHIGAN: Alger Co. (Au Train Falls); Delta Co. (Burn Bluff); Mackinac I.; Huron Mts.; Lleelenau, Ontonagon, Chippewa Cos.; Cheboygan Co.

(Douglas Lake); Ann Arbor; Washtenaw Co. (Oakland, see Darlington, 1938; Steere, 1947, etc.). WISCONSIN: Bayfield Co. (Mason and Cornucopia); Douglas Co. (Copper Creek, Black R., and Amnicon Falls); Iron Co. (Montreal R., Conklin, 1929). MINNESOTA: Carlton Co. (Carlton and Thompson); Chisago Co. (Taylors Falls); Cook Co. (Grand Marais, Schuster, 1953). IOWA: Mitchell, Winneshiek, Allamakee, Clayton, Dubuque, Jones, Manaska Cos. (Conard, 1954). MISSOURI: Boone Co. OKLAHOMA: Cherokee Co. (Little, 1936). KANSAS: Cherokee, Leavenworth and Woodson Cos. (*fide* McGregor, 1955). ARKANSAS: Shannon Co. (Pulltight Springs, n. of Eminence, Ozark Mts., *Anderson 12520a!, 12525!*); Washington Co. (Howards Bluff, e. of Springdale, *Anderson 12237*, trace among *Metzgeria conjugata!*).

The plant is distributed in the east almost indiscriminately at lower elevations in the Appalachian Plateau (to 4000 ft.) throughout the Piedmont, and the Coastal Plain. In the southeastern Coastal Plain it is replaced in southern Georgia and Mississippi by *C. contractiloba*, a very similar species.

The species was reported from Alabama (Barbour, 1903); no specimen has been seen. The Florida reports (Dade Co., *fide* Redfearn, 1952; Sanford, Orange Co., *fide* Haynes, 1915; Paradise Key in Dade Co., *fide* Frye and Clark, 1947) are all clearly errors, probably usually for *C. tuberculata* or *C. contractiloba*. (See Evans, 1938, p. 80, where the Sanford report is assigned to *C. contractiloba*).

ECOLOGY.—*C. biddlecomiae*, associated with its extensive distribution, shows wide variation in the choice of sites it inhabits. The species is almost indiscriminately corticolous and saxicolous in shaded, relatively damp sites.

At and near the northern edge of its range very commonly restricted to, or most frequent in, calcareous areas. In the ravines of Central New York, for instance, "the usual habitat . . . is on moderately dry calcareous sandstone rocks (often fossiliferous) which it covers with a fine, yellow-green overcast" (Schuster, 1949). Southward often similarly occurring, as near Pickwick Dam, Mississippi (*Schuster 19708*), where it occurs on shaded calcareous shale, and at Lake Waccamaw, N. C. (*Schuster 30021*) on coral limestone. However, as with many "calciphytes," the restriction to calcareous areas becomes much less marked at the southern periphery of its range, and the species occurs here on a variety of acidic rocks, as well as on the bark of a wide range of trees.

Northward on the bark of a variety of trees, particularly *Thuja occidentalis*, *Betula lutea* and sometimes even the highly acid *Tsuga canadensis;* also on the branches of yew, *Taxus canadensis*. Further southward showing almost no restriction as regards host trees, the species being observed on the bark of *Liquidambar styraciflua*, *Amelanchier* sp., *Tsuga caroliniana*, *Acer rubrum*, *Rhododendron maximum*, away from the immediate Coastal Plain, while within the latter on the bark of *Platanus occidentalis*, *Liriodendron tulipifera*, *Nyssa aquatica*, *N. sylvatica*, *Persea borbonia*, and even at the base of *Pinus taeda!* On bark associated with a very wide range of other Hepaticae, northward or at higher elevations chiefly: *Lejeunea cavifolia*, *Frullania asagrayana*, *Radula obconica*, *Lejeunea ruthii*, *Metzgeria conjugata;* the species here in damp sites. Southward the species is often markedly less restricted to humid and damp sites, and then may occur with *Porella platyphylloidea*, *Rectolejeunea maxonii*, *Radula caloosiensis*, *Lejeunea flava*, *Leucolejeunea unciloba*, *Leptocolea cardiocarpa*,

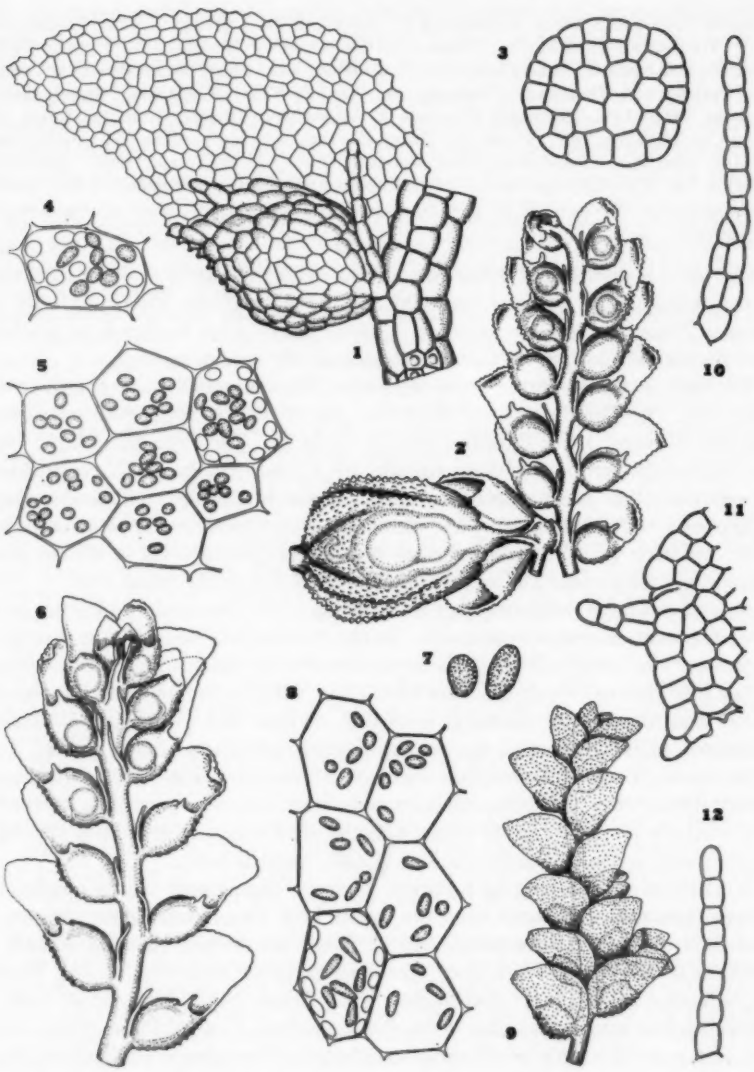

FIG. XIII. *Cololejeunea biddlecomiae* (Aust.) Evans. 1. Leaf and stem-sector, postical view (×165); 2. Fertile shoot, with androecium and perianth, the gynoecial innovation cut off at base (×54); 3. Gemma (×345); 4–5. Median cells (4, ×800; 5, ×665); 6. Androecial shoot, postical view (× *ca.* 70); 7. Oil-bodies (×2000); 8. Median cells (×670); 9. Shoot, antical view (× *ca.* 35); 10, 12. Styli (×270); 11. Apex of lobule (×270). (Figs. 1–2 Virginia, *Schuster 34521;* 3, North Carolina, *Schuster 28682;* 4–5, 7, Turkey Run State Park, Indiana, *Schuster 18325;* 6, 9, Mouth of Seneca, W. Va., *Schuster;* 8, Hemlock Bluff, Cary, N. C., *Schuster;* 10–12, Lake Drummond, Va., *Schuster 34529*).

Cololejeunea minutissima, *Leucolejeunea clypeata*, *Metzgeria furcata*, *Frullania inflata*, *F. squarrosa*, *F. eboracensis*, *F. kunzei*, and *F. brittoniae*.

DIFFERENTATION.—*C. biddlecomiae* is a species showing only a slight degree of variation, and never closely approaches our other species of the genus.

Superficially it is perhaps most similar to *C. contractiloba* (a species with an almost exactly complementary distribution in the extreme southeast). The type of gemmae (without slime cells on the margins, and without strongly protruding marginal cells), the coarsely tuberculate antical lobes but smooth postical lobes, and the smooth, very faintly segmented oil-bodies all suggest a clear affinity of the two species. However, the presence of distinct, filiform styli, and the presence of a prominent, usually 2-celled apical tooth of the lobule (on mature leaves) serve to separate *C. biddlecomiae* at once from *C. contractiloba*. *C. biddlecomiae* also has the leaves almost uniformly provided with inflated lobules (Fig. XIII: 6), while *C. contractiloba* is normally strongly heterophyllous. The gemmae of *C. biddlecomiae* are also formed, at maturity, of more numerous cells (Fig. XIII: 3), and appear always to be restricted in development to the postical face of the leaf lobe. The smooth oil-bodies, and the generally 2-celled apical tooth of the lobule also suggest a more distant affinity to *C. diaphana*, a tropical and subtropical species. Furthermore, very occasionally phases of *C. biddlecomiae* are found in which the cells of the antical lobe are only slightly tuberculate-produced, much as in "normal" phases of *C. diaphana*. *C. diaphana* differs at once, however, in the markedly heterophyllous leaves, the more attenuate and ovate-lanceolate leaves, and the very different, fewer-celled gemmae, whose margins are angulate-produced, and which bear 3 gibbous cells.

The presence of a distinct stylus (Fig. XIII: 10, 12) suggests the much smaller species, *C. ornata*. This species differs from all forms of *C. biddlecomiae*, however, in the tuberculate postical faces of the normally developed lobules and in the often tuberculate-armed and polymorphous styli, which may appear denticulate. The "tubercles" of the cells are also very high and prominently thickened in *C. ornata*, while those of *C. biddlecomiae* are medium high and slightly thickened at their tips. Finally, gemmae (Fig. XIII: 3) are very freely produced in *C. biddlecomiae*, while they are unknown in *C. ornata*. *C. biddlecomiae* is the only species of the genus (in our area) to show a strict restriction of the gemmae to the under surface of the antical lobes. In *C. diaphana* the gemmae are usually similarly situated, but occasional ones develop from marginal cells.[3]

[3] Frye and Clark (1947, p. 876) state "Gemmae unknown." However, Evans (1938, p. 78) correctly notes that they are "often produced in abundance." Furthermore, at least one of the "Examinations" of *C. biddlecomiae* listed by Frye and Clark (*Blomquist 8742*) shows free development of gemmae. The illustrations, taken from Pearson, fail to show the true form of the lobule. They figure a "female bracteole," a structure which does not exist in *Cololejeunea*. They also state that the inflorescence may be "unisexual," a condition never present in any specimen I have seen (Fig. XIII: 2). The female inflorescence is described as "on a main shoot" whereas it is normally on a very abbreviated lateral shoot (Fig. XIII: 2). The male bracts are described as 1-2-androus, whereas all that have been seen by the author are uniformly 1-androus.

Stevens (Bull. Torrey Bot. Club **37**: 366–369, fig. 1, 1910) has also described the origin and segmentation of the gemmae in this species in detail.

Although *C. biddlecomiae* exhibits only tenuous relationships with the three aforementioned regional species of *Cololejeunea*, it is very closely allied to the common, suboceanic European *C. calcarea* (Lib.) Spr. Evans (1902) and Pearson (1890) both discuss the relatively slight differences between these two species. A valid case can probably be made in favor of regarding the American *C. biddlecomiae* as only a subspecies of the European *C. calcarea*.

Whatever the taxonomic status applied to the American plant, it is easily separated from the European *C. calcarea*. *C. biddlecomiae* has more ovate leaf-lobes which are merely acute or subacute at the apices (Fig. XIII: 1, 6); in *C. calcarea* the lobes are ovate-lanceolate and commonly strongly attenuated to acuminate distally, with the deflexed tips usually formed by 2 elongated, superimposed cells. The styli of *C. biddlecomiae* are typically as much as 7–12 cells long, whereas those of *C. calcarea* are usually 2–4 cells long. The lobules of *C. biddlecomiae* are also somewhat smaller, as compared with the lobes, averaging scarcely more than one-fourth the area of the lobes; in *C. calcarea* the lobules appear more strongly inflated and are one-third to one-half the area of the lobes. Finally, the cellular protuberances, or "tubercles," are less coarse in *C. biddlecomiae* than in *C. calcarea*. Indeed, in many of the more mesic phases of *C. biddlecomiae* (such as *RMS 36048*, near Tryon, N. C.) the tubercles of the lobes are very low, their tips are hardly thick-walled; in such cases they may easily be overlooked.

Cololejeunea ornata Evans

Cololejeunea ornata Evans, The Bryol. **41:** 73, figs. 1–20, 1938.

Forming small, *pure- to whitish-green patches*, often growing over more robust hepaticae or mosses; shoots prostrate, *extremely small and delicate*, 250–350 μ wide, occasionally up to 420 μ in width. Stems irregularly, sparsely branched, usually less than 2 mm. in length × 36–45 μ in diam. Leaves slightly imbricate, obliquely spreading, *ovate-pointed*, (160) 180–200 (235) μ long × 100–120 (135) μ wide (but often less robust), convex and with the *acute apices* usually distinctly deflexed; apices sharp, ending in a single cell, *or 2 superimposed cells;* postical base somewhat ampliate, extending across the stem; lobe surfaces *strongly roughened* and margins irregularly but sharply crenulate-denticulate from conically protuberant cells. *Lobules strongly heteromorphic, only a minority inflated and fully developed*, the large majority variously reduced; maximally developed lobules oblong-ovate, *ca.* 100 μ long × 80 μ wide, with keel somewhat inflated (and strongly denticulate with projecting cells); free margins of well-developed lobules somewhat involute near base, flattened distally, the apex formed (under optimal conditions of development) by an *apical tooth formed of 2 superimposed cells;* proximad of apical tooth (1) 2 small 1-celled proximal teeth project, separated from each other by single cells that are not protuberant; abaxial (external) surface of *lobule with cells, with isolated exceptions, bearing tubercles* (but reduced lobules usually with only carinal tubercles, the surface tubercles often reduced or absent). *Styli distinct, prominent*, filamentous, 4–7 cells long, uniseriate throughout or occasionally locally biseriate; isolated to *occasional stylar cells tuberculate-produced*, the styli thus in part denticulate in outline. Styli often smaller, especially on weakly developed plants, then 2-3-celled, and uniformly formed of non-projecting cells, or rarely even reduced to an ephemeral slime papilla. *Cells very small*, the marginal *ca.* 8–9 μ, locally 10–11 μ, the median 9–12 μ and subisodiametric to 11–15 × 15–19 μ and polygonal, extremely leptodermous and with vestigial to small trigones, but the very salient tuberculate protuberances strongly thickened at their apices. Oil-bodies mostly *3–5 (6) per cell*, subglobose and 2 × 2–3 μ to

fusiform or ellipsoidal and 1.8 × 4 to 2–2.2 × 5–6 μ, *extremely smooth and appearing almost homogeneous* (the spherules hardly distinct, even under oil-immersion); chloroplasts *ca.* 3–3.5 μ long. Gemmae unknown.

Autoecious. *Androecial branches essentially identical with normal vegetative branches*, often proliferating vegetatively at apex; bracts loosely imbricate, in 2–4 pairs; lobes similar to those of vegetative leaves, similar in size; lobules large, inflated, with arched keels, the free margins with apical tooth usually less marked, only 1-celled, scarcely more evident than other juxtaposed projecting cells (the margin of apex of lobule thus sparingly denticulate); styli well-developed, as in vegetative leaves; bracts monandrous. Gynoecia always on *very abbreviated lateral branches*, bearing a single rudimentary leaf below the bracts. Bracts *much broader than long*, sheathing base of perianth, suberect, obscurely complicate, the keel rounded; lobes similar to leaf-lobes, but lobules highly modified; lobules variable, varying from *ca.* 160 μ long × 90–120 μ wide, free for less than 0.35 × their length, often emarginate or 2–3-lobed at apex (outer bract) to somewhat larger and broader, entire at apex (inner bract). Outer bract with distinct, prominent, variable stylus, inner bract lacking stylus; margins of lobes and lobules of bracts distinctly crenulate-denticulate, like leaf-margins, the abaxial faces, except near base, with cells tuberculate (like lobe surfaces). Perianth obovoid from a cuneate base, abruptly narrowed into a very short but discrete beak, *ca.* 300–350 μ in length × 250–300 μ in subapical diam.; upper third with 5 rather weakly-marked keels; entire surface of perianth, except at the base and the beak, with cells strongly tuberculate-produced.

TYPE: Over mosses on face of dolomitic rocks, 2 mi. e. of Coal Creek, Anderson Co., Tennessee (*A. J. Sharp 63*, March 1, 1931); a portion of the type in the Tennessee herbarium has been studied.

DISTRIBUTION.—Evidently a limestone species of very restricted range, appearing to occupy two centers, the phytogeographically "old" Appalachicola River region of western Florida (the *Torreya-Taxus floridana* region), and the even older limestone region of the western edge of the Appalachian Plateau, in Tennessee. The Alachua and Citrus County reports perhaps mark a third, "Oligocene Island," center. Although known from very few stations, the species is readily recognizable as one of the so-called "old tropical species."

The plant is not only very local, but appears to occur in only small quantity at the stations where it has been found. It often occurs as very small, inconspicuous patches as an epiphyte, and is readily overlooked on account of its very small size.

Tennessee: ANDERSON Co. (Two mi. e. of Coal Creek, *A. J. Sharp 63, 65, 34145*, 1931, 1934). **Florida:** JACKSON Co. (Chipola R., *Kurz and Kennedy 145;* Dothan Highway, 8 mi. n.w. of Marianna, *Kurz 269*); ALACHUA Co. (Buzzards Roost, 7–8 mi. w. of Gainesville, *RMS 33348a, p.p.*); CITRUS Co. (Lime sinks near Pineola, *RMS 37224, p.p.*).

ECOLOGY.—Apparently confined to limestone and dolomitic rocks, where the species may occur growing over robust mosses, rather than directly on rock itself. The plant is not strongly mesophytic, being reported from an "xerophytic limestone cliff," but southward occurs largely in limestone grottoes, in humid sites.

At the Alachua station, the first reported since the description of the species, the *C. ornata* is epiphytic on *Dumortiera hirsuta* on a coral limestone cliff, associated with *Cololejeunea contractiloba*, *Lejeunea calcicola* and traces of *L. minutiloba*. Associated also are the tropical ferns, *Asplenium abscissum*, *A. verecundum*, *A.*

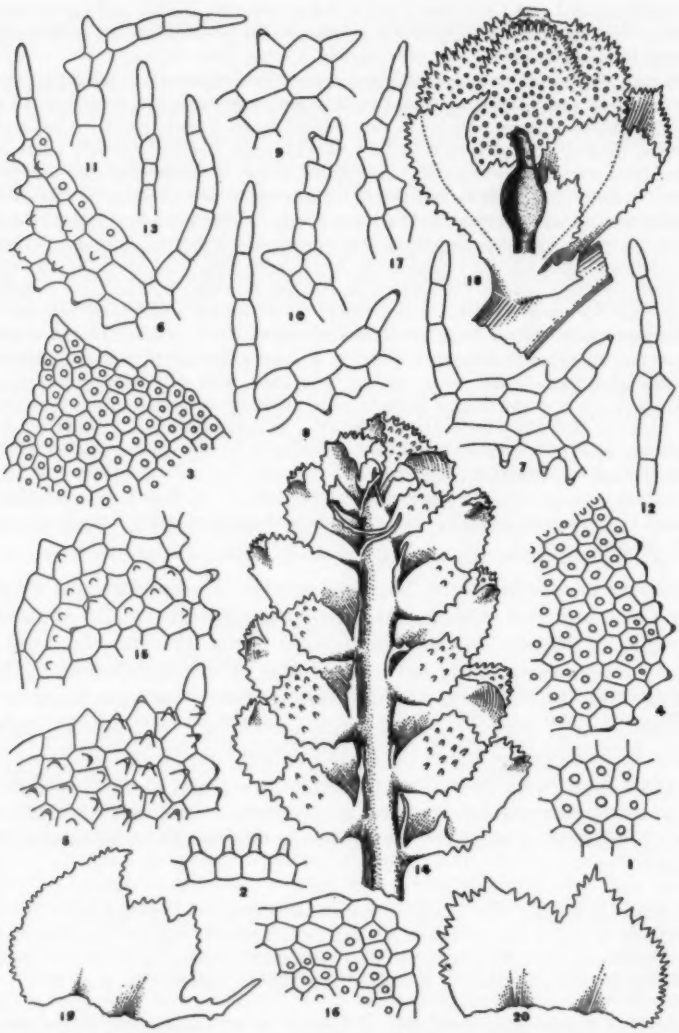

FIG. XIV. *Cololejeunea ornata* Evans (From Evans, 1938). 1. Cells of leaf-middle (×300); 2. Optical section of median cells of lobe (×300); 3. Apex of lobe (×300); 4. Dorsal base of lobe (×300); 5. Apical part of well-developed lobule (×300); 6–9. Poorly-developed lobules, with styli drawn in, except in Fig. 9 (×300); 10–11. Styli of well-developed lobules (×300). 12–13. Styli of poorly developed lobules (×300); 14. Androecial branch (×100); 15–16. Apical portions of lobules of male bracts (×300); 17. Stylus of male bract (×300); 18. Perianth and bracts, postical view (×100); 19–20. Exterior and interior bracts, respectively, of another inflorescence (×100). (Figures all drawn from the type collection).

cristatum, *A. pumilum*, and *A. resiliens*, as well as *Goniopteris reptans*. More recently found in Citrus County, again growing over *Dumortiera hirsuta* and *D. nepalensis*, associated with many of the above species (*C. contractiloba*, *L. minutiloba*, *A. resiliens*, *A. abscissum*, *A. verecundum*, *G. reptans*) as well as with the more "southern" *Tectaria heracleifolia* and *Adiantum tenerum*, and with *Asplenium heterochroum*.

DIFFERENTIATION.—*C. ornata* shows immediate relationships to both *C. tuberculata* and *C. biddlecomiae*. It agrees with the first in the very small size, extremely salient tubercles produced by the leaf cells (Fig. XIV: 2), the ability of the cells of the lobules to develop similar tubercle-like outgrowths (Fig. XIV: 14), and in the highly polymorphous leaves, with many of the leaves provided with more or less reduced lobules. *C. ornata* is at once separable from *C. tuberculata* by the somewhat beaked perianth, the much broader than long female bracts, one of which, at least, bears a prominent stylus (Fig. XIV: 19), and by the development of distinct styli of the vegetative leaves and perigonal bracts (Fig. XIV: 14).

In the prominent styli, as well as in most other features, *C. ornata* exhibits a very close affinity to *C. biddlecomiae*. It differs from the latter, much more ubiquitous species, in a series of features, among them (a) the much inferior size; (b) the much more polymorphous lobules (Fig. XIV: 6–8, 14), with only a small minority large and inflated; (c) the tuberculate-produced cells of well-developed lobules; (d) the occasional denticulation of the styli (Fig. XIV: 10–13), isolated cells of which are tuberculate-produced; (e) the much more salient, high tubercles of the cells; (f) absence of gemmae; (g) somewhat sharper and often somewhat apiculate dorsal lobes.

The examination of material of *C. ornata* is often complicated by the sparsity of the plants, but more so by the fact that most of the plants give the impression of being poorly developed. Only very occasional leaves bear fully developed and inflated lobules (Fig. XIV: 5), in which we can see that the apical tooth is fundamentally 2-celled, as in the related *C. tuberculata* and *biddlecomiae*. Such lobules generally bear 2 very weakly-marked preapical teeth of the free margin; these teeth represent merely cells that are tuberculate-produced in the plane of the lobule, separated from each other (and from the apical tooth) by single cells that do not project marginally. However, these latter cells, as well as other lobular cells of mature lobules, are conically armed or tuberculate (Fig. XIV: 5). The only exceptions are found in the 1–2 rows of cells bordering the free margin of the lobule, proximal of the apical region of the lobule. Evans (1938, p. 76) states that the hyaline papilla is proximal in position; it could not be demonstrated in the sparse material available for study.

The majority of lobules show various levels of reduction in size, but often show scarcely any reduction in complexity. A series of such lobules is shown, illustrating progressive reduction in size and complexity, in Fig. XIV: 6–9. When moderately reduced (Fig. XIV: 6) the surface cells may remain tuberculate, and an elongated, 2-celled apical tooth may be present, although no preapical teeth may occur. In cases where the lobule is scarcely more reduced

Fig. XIV: 7), an apical tooth is present, and may be 3 cells long, as well as a single preapical tooth, although the lobular cells are smooth in this case. In extreme cases (Fig. XIV: 8–9) the apical tooth may be 2-celled or only 1-celled, and a single preapical tooth may be present (Fig. XIV: 9) or absent (Fig. XIV: 8). Associated with reduction of the lobules, the styli are usually shorter, averaging only 4–5 cells long, are uniseriate throughout, and do not show the characteristic tendency for some cells to become tuberculate-produced. This high level of polymorphism in development of the lobules does not recur in any other North American species of the genus.

In the absence of gemmae, the correct position of this species in the sections here established is a matter of conjecture. If the gemmae prove to possess swollen rhizoid initials, the species may be considered to be allied most closely to *C. tuberculata* (Sectio Diaphanae), but if the gemmae prove to be smooth-margined and lacking "organs of attachment" the plant will be better referred to the Sectio Calcareae, and placed in close juxtaposition to *C. biddlecomiae*.

The practical problem of identification of material of this rare species is complicated by the fact that most plants give the impression of being merely a juvenile phase of another species. The writer's collections from Citrus and Alachua Counties are a case in point. The plants in those collections are extremely sparse, and occur amidst more frequent plants of an extreme phase of *C. contractiloba*. The *C. ornata* at first appears to be a juvenile phase of the latter species, averaging only 250–320 μ, rarely up to 400 μ, in width (*vs.* 350–420 μ for the *C. contractiloba*). Except for the size difference, the two species are almost totally identical in appearance, in antical aspect, both having ovate-pointed leaves, and both possessing extremely sharply tuberculate, almost hispid lobes. In postical aspect two differences are evident. The *C. ornata* has the lobules extremely variable, ranging from 2–3-celled to 8–10-celled, with the larger bearing a sharp, elongate, 2–3-celled apical tooth (in the plants seen not a single lobule was large and inflated, and almost none bore the tuberculate extensions of the cells); in *C. contractiloba* the lobules are either normal and inflated, or reduced to a 2-celled vestige. In other words, the former species possesses heteromorphic leaves, the latter dimorphic ones. The second difference lies in the styli. Those of *C. ornata* are distinct, usually at least 4–5 cells long (but in the writer's collection sometimes only 3-celled; in no case are any cells produced); those of *C. contractiloba* are reduced to a vestigial slime papilla.

The Citrus County material is even more critical in that the plants only very rarely have isolated, large lobules, and these usually lack conically-projecting cells except near the keel, and along the free margins of the lobule. Furthermore, the styli are not uniform in occurrence, but are sometimes reduced to an ephemeral slime papilla, in other cases only 2–3 cells long, rarely formed of four superimposed cells. In no case were the cells of the styli conically protuberant. Such plants, with reduced styli, are quite subject to confusion with *C. tuberculata*. One feature of the Citrus County plants that at once allows their attribution to *C. ornata*, rather than to *C. tuberculata*, is the form of the female bracts. These are very broad, flattened out much broader than long, and subequally bilobed, with the lobes ovate to broadly ovate.

SECTIO DIAPHANAE sectio nova

Propagulae aliis cellulis marginalibus quasi acutis angulis productis, in margine plerumque tres tumidae rhizoido-initiales cellulae; folia valde heterophyllosa, apice acute; cellulae quasi argute tuberculos productae.

Plants commonly markedly dimorphic (Fig. XVI: 7; Fig. XVII: 6); leaves with lobes ovate-lanceolate and pointed, length 1.5–2.5 × width. Lobules, when inflated, always with a 2-celled apical tooth lying in the plane of the lobule; stylus absent. Cells of lobe (in *C. tuberculata*, also of lobule) conoidally produced, at least the lobe distinctly roughened (in *C. diaphana* often slightly or scarcely so, in extreme shade forms); trigones often distinct; oil-bodies delicately segmented, appearing fine-papillose or almost homogeneous (Fig. XVI: 1, 6); ocelli lacking. Gemmae arising from postical face of lobe, rarely from marginal cells, specialized: formed of 16–22 cells, of which 4–6 protrude sharply and angularly, while (2) 3 are swollen, forming slime-cells or rhizoid-initials (Fig. XVII: 8).

Perianths with keels sometimes armed with protuberances or teeth, but not strongly dorsiventrally compressed, their cells tuberculate-produced.

The Diaphanae are a well-defined complex, characterized essentially by (a) marked heterophylly; (b) tuberculate cells of the lobes; (c) specialized gemmae, similar to those of *Aphanolejeunea*. This complex, in these three characters, closely approaches certain species of *Aphanolejeunea*. The specialized gemmae alone suffice to separate the members of this group from all our other species of *Cololejeunea*. The only regional members are the two following species, *C. tuberculata* and *C. diaphana*.

Cololejeunea tuberculata EVS.

Cololejeunea tuberculata Evans, The Bryol. **18** (5): 84, figs. 1–9, 1915; Evans, *ibid* **41**: 80, 1938; Frye and Clark, Univ. Wash. Publ. Biol. **6** (5): 874, figs. 1–10, 1947.

Plants *minute and delicate*, pale green, with age sometimes almost decolorate, in small patches or scattered, the shoots 300–360 μ wide. Stems 30–35 μ in diam., irregularly, sometimes freely branched, the branches widely spreading; usually 1 subfloral innovation. Leaves slightly distant, contiguous, or barely imbricate, spreading at an angle of 45–65°, plane or barely convex, but often with apices of lobes deflexed. *Lobes ovate-pointed*, up to *180–210 μ long* × 120–135 μ wide, often smaller, gradually contracted to an *acute or subacute*, rarely obtuse apex terminating in a solitary cell; both antical and postical margins moderately arched proximally, nearly straight distally, sharply denticulate from strongly projecting cells; surface of *lobe strongly tuberculate throughout. Lobules polymorphous, very often obsolete* (particularly in conjunction with fertile branches), when well-developed inflated and ovate, up to 95 μ long × 75 μ wide; *apex* of inflated lobules normally terminated in an apical tooth formed of *2 superimposed cells*, the hyaline papilla at base of the proximal of these two teeth, on its inner surface; proximal tooth shorter, usually obtuse or blunt, usually separated from apical tooth by a sinus formed by a single cell; *cells of inflated lobules, or most of them, strongly tuberculate produced; stylus vestigial*, an ephemeral slime papilla. Cells thin-walled and with vestigial trigones, but *drawn out into a high, prominent, apically rounded* conoidal tubercle, whose apex is thick-walled; marginal cells *ca.* 7–8 μ; median cells 8–9 × 9–12 μ; basal cells 8–10 × 14 μ. Asexual reproduction by discoid gemmae, which are *ca.* 50 × 60 μ, composed (at maturity) of *16 cells, the margins angulate* owing to 5–6 protuberant cells; *with 2 (or 3?) swollen slime cells.*

Autoecious. Androecia terminal (evidently not proliferating vegetatively) on a short lateral or more or less elongate branch, *ca.* 200–260 μ wide; bracts in 2–4 pairs, similar to leaves but usually somewhat smaller, with lobes less obliquely spreading, and with apical teeth of lobules poorly developed; monandrous. Female inflorescence on a more or less abbreviated lateral branch, with a solitary innovation (the innovation short and sterile or

obsolete). Bracts obliquely spreading, weakly complicate, the lobe ovate-lanceolate, *acute at the apex*, which is often inflexed, 200–250 μ long × 70–90 μ wide; margins and surface coarsely tuberculate as on the leaves. Lobule narrowly ovate to ovate-oblong, free for less than 0.4 × its length from lobe, 160–180 μ long × 60–70 μ wide, variable at the apex (from acute to truncate), irregularly denticulate, the surface more or less tuberculate (at least adjacent to keel). Perianth obovoid, *ca.* 300 μ long × 220 μ wide near the apex, obtusely 5-angled near apex when juvenile but *strongly inflated and essentially ecarinate at maturity, without a beak*, its surface to below the middle sharply tuberculate from projecting cells. Capsule *ca.* 150 μ in diameter.[4]

TYPE: Over living leaves of *Trichomanis krausii* Hook. et Grev., Nixon-Lewis Hammock, Everglade Keys, Dade Co., Florida (*J. K. Small and C. A. Mosier 5250*, NYBG!).

DISTRIBUTION.—Known only from a restricted region of southern Florida (Dade Co.), in the so-called Everglade Keys, and from a few stations in the Oligocene Island area of northern Florida, westward to the Tallahassee region of western Florida. As a consequence of the minute size, and very scattered occurrence, the species has been but very rarely collected. It will almost certainly be found to occur in the West Indies.

Florida: DADE CO. (Nixon-Lewis Hammock, *Small and Mosier 5250*, type!; Sykes Hammock, Everglade Keys, *Small, Mosier and Small 5257!;* Hattie Bauer Hammock, Everglade Keys, *Small and Mosier 6005!;* Timms Hammock, Everglade Keys, *Small and Mosier 6004!*). ALACHUA CO. (Gainesville, *McFarlin 1866;* along creek s. of Athletic Field, Gainesville, *Little*). LEON CO. (Four miles w. of Tallahassee on Old Spanish Trail, *Kurz and Little*).[5]

ECOLOGY.—The original material of this rare species was collected on the living leaves of filmy ferns, *Trichomanis krausii* and *T. sphenoides*, in deeply shaded damp hammock forests. The species appears to be extinct in the hammocks from which Evans (1915) first described it. No information on the sites of the species in northern Florida is available, but the plants were not epiphyllous.

DIFFERENTIATION.—*C. tuberculata* rivals in delicacy and minuteness the very similar *C. ornata*, which has also been found in northern Florida. *C. tuberculata* agrees with *C. ornata* also in many other respects, indicating a close affinity; among them are: (a) the very prominent development of the tubercles of the leaves (Fig. XV: 8), which occur on both the external faces of the lobes and lobules (Fig. XV: 3–4); (b) the ovate-pointed dorsal lobe; (c) the strongly tuberculate perianths (Fig. XV: 1); (d) the similar, dimorphous lobules, with a high incidence of obsolete lobules (Fig. XV: 2, 3); (e) similar leaf cells. However, two important distinctions occur: in *C. tuberculata* the styli are absent (Fig. XV: 3), in *C. ornata* well-developed, and in *C. tuberculata* the perianths are beakless (Fig. XV: 1), while they bear a distinct but low beak in *C. ornata*. These distinctions appear sufficient to warrant a specific separation.

[4] Preceding description in large part modified from Evans (1915).

[5] I have spent some time in carefully studying a specimen (NYBG) determined by Dr. A. W. Evans as *C. tuberculata* Evs., from Annuttalagga Hammock, Hernando Co., Florida (E. and R. St. John and J. K. Small, April 10, 1936). No trace of *C. tuberculata* was found, but small amounts of *C. diaphana cristulata* and *C. contractiloba* were found. It is probable that the latter served as the basis for the report of *C. tuberculata*. However, these plants had the typical postically displaced, obscure, 1-celled apical tooth of *C. contractiloba*.

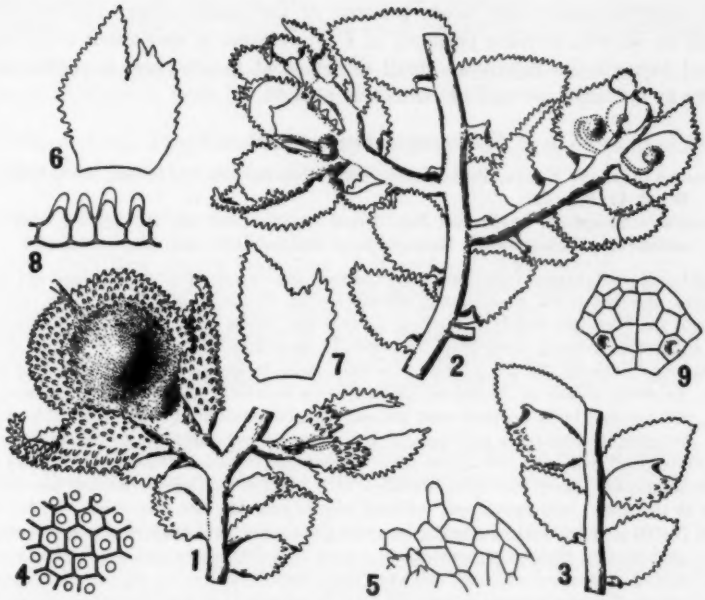

FIG. XV. *Cololejeunea tuberculata* Evans (from Evans, 1915). 1. Part of plant, with perianth and an unfertilized gynoecium, postical aspect (×100); 2. Part of another plant, with gynoecium and androecium, postical aspect (×100); 3. Part of sterile shoot, postical aspect (×100); 4. Median cells of lobe (×300); 5. Apex of well-developed lobule (×300); 6–7. Bracts from a single inflorescence (×100); 8. Cells from lobe of a bract, optical section (×300); 9. Gemma (×300). (Figs. 1, 3–9 from the type specimen; Fig. 2 from Sykes Hammock, Dade Co., *Small 5257*).

C. tuberculata also bears some resemblances to *C. biddlecomiae* and *C. contractiloba*, two species with distinctly tuberculate cells of the ovate-pointed dorsal lobes. However, in both of these larger species the cells of the lobules are uniformly smooth. A more fundamental distinction lies, perhaps, in the gemmae. These are angulate-margined, bear "slime" cells, and are only 16-celled in *C. tuberculata* (Fig. XV: 9); by contrast, in *C. biddlecomiae* and *C. contractiloba* the marginal cells are never angularly produced, "slime" cells are lacking, and the mature gemmae are formed of 18–20 or more cells. In the extremely strongly developed tuberculate extensions of the cells of the lobes (Fig. XV: 8) *C. tuberculata* much more closely approaches *C. ornata* than *C. biddlecomiae* and *C. contractiloba*. Both of the latter species are much less strongly roughened, the conoidal tubercles being low and rounded with the wall relatively weakly thickened at the apex of the tubercle.

Evans (1915) also pointed out that the rare Oceanic-Mediterranean *C. rossettiana* (Massal.) Schiffn., of Europe, bears tubercles on the lobules. This species further agrees with *C. tuberculata* in the obsolete stylus. However, *C. rossettiana* is a somewhat larger plant, the lobules of which are irregularly dentate

on the margins, and which bears gemmae of the dorsal lobes. The somewhat obovoid to obovoid-clavate perianth of *C. rossettiana* is also much more longly exserted beyond the relatively small perichaetial bracts, and is distinctly 5-carinate at maturity, as well as obsoletely beaked.

Cololejeunea diaphana Evans

Cololejeunea diaphana Evans, Bull. Torrey Bot. Club **32:** 184, pl. 5, figs. 9–14, 1905; The Bryol. **41:** 80, 1938.

Cololejeunea subdiaphana Jovet-Ast, Rev. Bryol. et Lich. n.s. **16:** 28, fig. VI, 1947 (new synonymy); Schuster and Hattori, Jour. Hattori Bot. Lab. No. 11: 65, 1954.

Bright yellowish-green, forming small prostrate patches or occurring as isolated creeping plants; shoots 410–500, occasionally 550–600 μ wide. Stems 30–38 (46) μ in diam., sparsely branched, the branches widely spreading. Leaves flat, when moist or dry closely flattened to the substrate, *strongly dimorphic*, horizontally spreading at an angle of 45–70°, distant to contiguous; lobe *ca.* 250–270 μ long × 75–150 μ wide, in length 1.8–2.5 × length of larger lobules, narrowly ovate to *lanceolate, pointed*, very narrowly and nearly transversely inserted, not falcate, both postical and antical margins somewhat curved, or the postical nearly straight; lobe-margins and usually keel more or less crenulate or denticulate from tuberculate-produced cells; *lobe-apices acute to subobtuse, terminated by a solitary cell usually. Lobules dimorphic, many (or most) reduced to 1–2-celled rudiments* bearing the hyaline papilla at the apex; well-developed, inflated lobules smooth, *non-tuberculate*, *ca.* 100–150 μ long × 70–100 μ wide, with a slightly to strongly arched keel that is either smooth and barely crenulate or distinctly denticulate owing to tuberculate-produced cells; apex of lobule bearing a rounded, solitary cell near apex (proximal tooth), separated by one cell from a *2 (rarely 3)-celled straight distal tooth*,[6] which lies nearly midway between the proximal tooth and the keel; hyaline papilla entally displaced, at base of the 2-celled apical tooth, situated usually *slightly nearer sinus between proximal and distal tooth*, than near sinus between distal tooth and keel (thus fundamentally proximal; but sometimes almost perfectly median in position between the two sinuses). Leaf cells *ca.* 10–13 μ marginally, *ca.* 11–13 (15–19) × 17–20 (22–25) μ medially, to 10–16 × 28–30 μ basally, thin-walled and with minute trigones to somewhat thick-walled and with distinct trigones and occasional vague intermediate thickenings; oil-bodies appearing papillose, *very finely segmented* (globules < 0.8 μ), ovoid to ellipsoid to fusiform, small (2.5 × 3 to 3 × 5–6 μ to 2 × 7.5 μ), (2) 4–6 per median cell. Asexual reproduction by discoid gemmae arising on the lower (and sometimes upper) lobe surface (*more rarely also marginally!*); gemmae usually 20-, more rarely 19- or 22-celled, with 3 globose slime-cells, *ca.* 75 × 90 μ (in var. *cristulata* 15-16-celled and 60 × 65–70 × 90 μ).

Autoecious. Male inflorescence on a short lateral branch devoid of normal leaves, or on an elongate leafy shoot; bracts in 1–6 pairs, subimbricate to distant; lobes suberect, lanceolate, acute, *ca.* 130 μ long × 60 μ wide; lobule somewhat concave, bluntly pointed, *ca.* 80 μ long × 50 μ wide; antheridia solitary. Female inflorescence either on a short, leafless branch or on a main branch, with 1 subfloral innovation; ♀ bracts erect-spreading (more widely so after fertilization), complicate, the lobe narrowly oblong to lanceolate, *ca.* 280–400 μ long × 80–100 μ wide, rounded to acute apically; lobule oblong to cuneate, *ca.* 120 μ long × 35 μ wide (less than one-half the length of the lobe). Perianth obpyriform, distally sub-

[6] Evans (1905) originally described this tooth as short and blunt, and figured it as 1-celled. However, later (Evans, 1938) he clearly stated that it was 2-celled. Frye and Clark (1947) describe it as "a mere projecting cell, short, blunt." In my experience, it is virtually invariably elongate, 2 full cells in length, rarely of 3 cells in a row. The very isolated instances where the apical tooth is 1-celled (Fig. XVII: 2) clearly represent an abnormal condition.

truncate (except for the beak), *ca.* 300 μ long × 200 μ wide near apex, smooth (except for the carinae); dorsal face flat, lower face with a short carina; lateral keels denticulate; beak short.

TYPE: "In hammocks near the homestead trail, between Cutler and Camp Longview" [southeast of Miami, in Dade Co., Florida.] (*Small and Carter 1365, p.p.*).

DISTRIBUTION.—Restricted to the tropical and subtropical portions of Florida, but extending north and west of Gainesville.

Florida: DADE CO. (Hammocks near Old Cutler Road, s.e. of Miami, *Small and Carter, type*); ALACHUA CO. (Gainesville, *Nelson and Little*); SEMINOLE CO. (Sanford, *Rapp*); CLAY CO. (Gold Head Branch State Park, *R.M. and O.M. Schuster 31407, 37207b, 31404*); DADE CO. (Mathesson Hammock, *RMS 22692* [*Plate*], *22680, 33976;* Costello Hammock, near Silver Palm, *RMS 22932;* Ross Hammock, n. of Homestead, *RMS 33800a*, elobulate phase); HIGHLANDS CO. (Highlands Hammock State Park, w. of Sebring, *RMS 26011;* Sebring, *McFarlin*); Marion Co. (3–4 miles below Juniper Springs, on Juniper Creek, Ocala Ntl. Forest, *RMS 33450a, p.p., 33396;* Juniper Springs, Ocala Ntl. Forest, *RMS 31915*).

The plants from Clay County (*RMS 37207b*) occurred with *Aphanolejeunea evansii*. This collection extends the latter species to a new, limital point northward.

Otherwise known only from a single, doubtful report from Puerto Rico (Evans, 1911; 1938, p. 80), which acquires more probability with the recent extension of the species to Guadeloupe (sub. *C. subdiaphana*).

ECOLOGY.—Over living leaves in deeply shaded hammock forests, or (more frequently) on shaded bark or exposed roots of trees. In Florida occurring in the southern region on the exfoliating bark of Gumbo-limbo (*Elaphrium simaruba*) and other trees, such as *Xanthoxylum fagara, Simaruba glauca*, etc.; in central Florida over *Acer rubrum* and *Liquidambar styraciflua*. Undoubtedly a much wider range of host species occurs, both of smooth and rough-barked trees. On leaves (such as those of *Persea borbonia*) various other Lejeuneaceae are associated (*Leptocolea cardiocarpa, Cololejeunea minutissima, Lejeunea* cf. *longifissa*); on bark *Aphanolejeunea ephemeroides* is frequently associated (*RMS 26011, 31404*, etc.), but also *Rectolejeunea phyllobola* and *maxonii, Lejeunea laetevirens* and *bullata, Leptolejeunea elliptica, Lejeunea minutiloba* and *flava, Cololejeunea minutissima, contractiloba* and *subcristata* and *Frullania obcordata*. The species is evidently restricted more to shaded and humid sites than most other species of the genus from our area. On rough-barked trees often restricted largely to the fissures.

This species also rarely occurs on damp to wet decaying logs (as in Lake Co., Florida, *RMS 31510*); it occurs here as a peculiar form, with strongly tuberculate cells, which has an appearance very similar to *Aphanolejeunea evansii*; this variant is discussed subsequently. Associated under such conditions are *Lejeunea glaucescens* and *Riccardia cf. multifida*.

VARIATION AND DIFFERENTIATION.—This extraordinarily delicate species often has the appearance of a small and impoverished form of *C. subcristata*, to which it is probably only distantly allied. It is also very similar to impoverished or juvenile

plants of *Leptocolea cardiocarpa*. The type material, as well as the material collected by the author in Mathesson Hammock, is characterized by the virtual absence of conical elevations of the cells. The material from Sanford shows distinct development of tubercles of some cells of the distal portions of the lobe, and further differs in the presence of discrete trigones (occasionally somewhat bulging) and intermediate thickenings of the longer longitudinal walls. The plants from Clay County are similarly provided with conical tubercles of the marginal cells, as well as of the cells near the keel of the leaves (which is consequently crenulate in profile). Gemmae are often abundantly produced, and, in the material from Mathesson Hammock, arise not only from surface cells, but often arise from marginal cells as well, much as in *Aphanolejeunea*. Evans (1911, *1F*) first figured these from Puerto Rican material, but Frye and Clark, as late as 1947 still state "Gemmae wanting." If one compares the figure of the gemmae by Evans (1911) with that drawn from the material collected in Mathesson Hammock (Fig. XVI: 5), it is evident that the two are identical in form and number of cells. In both cases, each half of the gemma bears 6 or 7 peripheral or marginal cells, and usually 4 (more rarely 3 or 5) inner cells; either three or four quadrants may be cut off—as already noted by Evans—and the mature gemma is usually 20 (rarely 22) cells in size. The exact duplication of the features of the material collected by the writer in Florida, and that figured by Evans from Puerto Rico, suggests that the tentative report from the latter area is correct.

The gemmae have been studied extensively in the material from Clay County, Florida (*RMS 37207b*), in plants showing the markedly lanceolate lobes of the var. *cristulata*, but with the lobules averaging less than half the length of the lobes, on lobulate leaves. The gemmae in these plants usually consist of 20 cells, with each half formed of 6 peripheral and 4 inner cells. In such cases, the gemma bears 3 "slime" cells. This is also the case in the less frequent instances when a gemma bears 9 cells on one side, 10 on the other, and in those instances when there are 11 cells on one side, 10 on the other. However, in the few instances seen when the gemmae were of 22 cells, each half bore 2 "slime" cells. It deserves mention that in these plants, in distinction to some of the others studied, the gemmae appeared to be uniformly antical in origin, and confined to intramarginal cells. The taxonomic significance, if any, of these differences in ontogeny of the gemmae in *C. diaphana* is not clear at present.

All forms of the species which have been seen are characterized by the marked heterophylly (Fig. XVI: 7), reminiscent in many ways of *Aphanolejeunea*. In addition to this, *C. diaphana* has the hyaline papilla on the ental surface, at the base of the 2-celled apical tooth, *either midway between* the two sinuses, or slightly nearer to the sinus between apical and proximal teeth (Figs. XVI: 4, 8; XVII: 5, 6). The position of the hyaline papilla in this species throws serious doubt upon the generic value of the position of this papilla, in this case, and indeed upon the value of *Aphanolejeunea* as a genus.

The collection from Mathesson Hammock (*RMS 33976*) is quite deviant in the total suppression of lobulate leaves. In all cases the lobule is reduced to a 2-celled fold, the distal of the two cells bearing the hyaline papilla. Such plants, devoid of

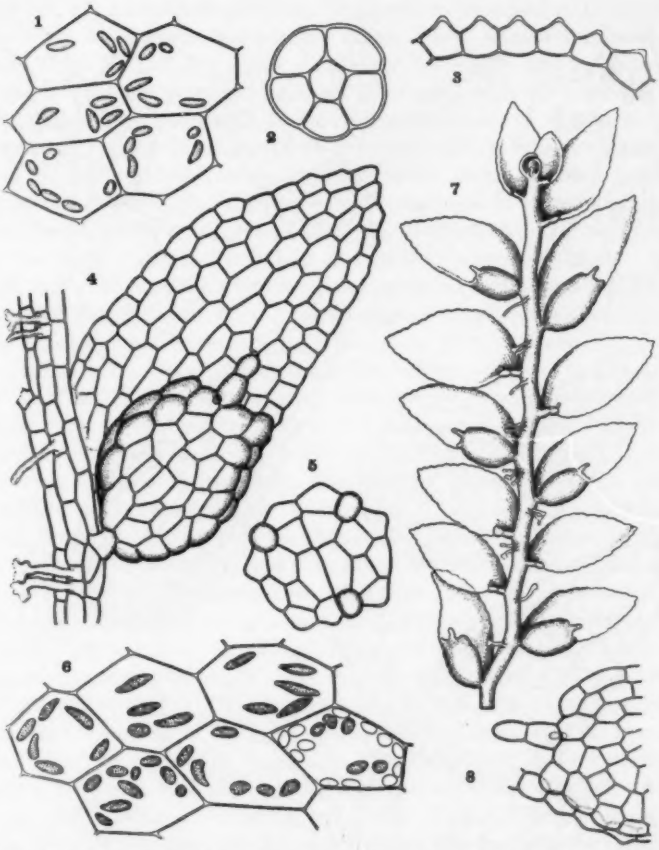

FIG. XVI. *Cololejeunea diaphana* Evans. 1. Median cells of lobe (×640); 2. Stem cross-section (×600); 3. Cells of keel, optical section (×330); 4. Leaf, leptodermous extreme (×285); 5. Gemma (×255); 6. Median cells, in right-hand cells the chloroplasts drawn in (×720); 7. Shoot, postical aspect, leptodermous phase (×80); 8. Apex of lobule, the hyaline papilla ental, indicated by stippling (×240). (Figs. 1, 4, 7. Mathesson Hammock, Florida, *Schuster 22692;* 2–3, 8, Gold Head State Park, Fla., *Schuster 31407* and *31407a;* 5, Florida, *Schuster 22680;* 6. Highland Hammock, Fla., *Schuster 26011*).

the occasional inflated lobules characteristic of the species, must be separated with care from *C. setiloba* (in which, however, the rudimentary lobule is marked at the apex by a 2–4-celled apical tooth). The elobulate phases of *C. diaphana* represent extreme shade forms, sterile, with very free development of gemmae; such plants totally lack the conical elevations of the leaf cells (thus approaching *C. subcristata*).

In the separation of *C. diaphana*, only *C. subcristata* and *C. contractiloba* need

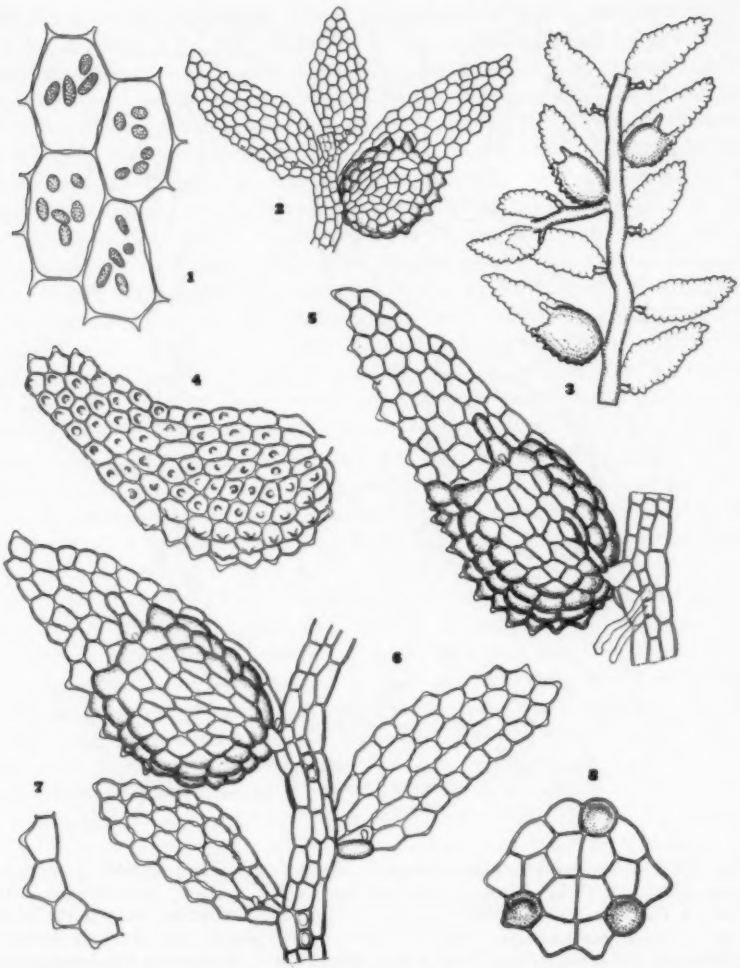

FIG. XVII. *Cololejeunea diaphana cristulata* Schuster. 1. Median cells (×640); 2. Apex of shoot, with 2 elobulate leaves and 1 lobulate leaf with atypical apical tooth (×135); 3. Sterile shoot, postical aspect (×78); 4. Leaf, antical aspect, with tubercles drawn in (×238); 5. Large leaf, postical aspect (×238); 6. Shoot-sector, postical aspect (×238); 7. Cells of keel, in optical section (×400); 8. Gemma (×430). (All drawn from *Schuster 31510*, from Alexander Springs, Lake Co., Fla.).

come under consideration. From the first, *C. diaphana* differs in the inferior size and greater delicacy, the very general obsolescence of many (though usually not all) lobules; the development, on at least the most robust leaves, of some marginal cells that are crenulate because of the tuberculiform protrusion of the wall

(Fig. XVI: 3, 4); the mature gemmae, 20–22-celled (16-celled in *C. subcristata*); the somewhat more lanceolate-pointed dorsal lobes, always terminated by a single cell (Fig. XVI: 4, 7). These differences are slight, and in leptodermous modifications that are poorly developed, great care must be exercised so that juvenile patches of *C. subcristata* are not misidentified for *C. diaphana*.

The generally slight development of tubercles (rarely well-developed on the basal half of the dorsal lobe), the generally narrower, more lanceolate (rather than ovate-lanceolate) leaves, and the very rare development of many inflated lobules all serve to separate *C. diaphana* from *C. contractiloba*. On well-developed lobules, the apical tooth is 2 cells long and relatively prominent in *C. diaphana* (much as in *C. subcristata*), while it is inconspicuous and only 1 cell long in *C. contractiloba*. The gemmae of *C. diaphana* and *contractiloba* are apparently identical in size in both, being usually formed of *ca.* 20 cells. Those of *C. diaphana*, however, differ in the development of swollen "slime cells."

Jovet-Ast (1947) describes as new *C. subdiaphana*, from Guadaloupe. This is admittedly "très proche du *C. diaphana*." It is supposed to differ from the latter by the more elongate leaves, the fewer bracts in the androecium (1–2 pairs), gemmae with the cells "un peu plus nombreuses" (formed of 19–21 cells), the apical tooth of the lobule "surtout . . . longue et à 2 cellules . . . ," and finally by the development of tubercles (when these occur at all) restricted to the basal one-half of the leaf. The figures and description of Jovet-Ast have been carefully compared with plants from somewhat xeric sites (base of *Liquidambar* trunk, Clay Co., Fla., *R. M. and O. M. Schuster 31407a*) which closely agree with *C. diaphana* and key to it in Evans (1938). These plants serve totally to break down the distinctions attempted above, since they show: (1) lobes with the distal cells usually tuberculate only on the marginal row, the tubercles usually dorsally displaced, so that the apices of the lobes do not appear denticulate; (2) leaves often with the cells of the basal region, near the keel and dorsad of the keel distinctly tuberculate; (3) lobule with apical tooth long and uniformly 2-celled, with a few exceptions, which show a 3-celled apical tooth. Evans (1905) states that the androecia of *C. diaphana* are formed of 1–6 pairs; this includes the range of 1–2 pairs given by Jovet-Ast for *C. subdiaphana*. The gemmae of *C. diaphana* are described by Evans (1911, p. 282) as 20–22 cells in size and figured as such (Fig. I, F); his figure closely matches those in Jovet-Ast (*l.c.*, Fig. VI, 17–18); the gemmae of plants from Mathesson Hammock (Fig. XVI: 5) are identical with those illustrated by Jovet-Ast for *C. subdiaphana*. The leaves of *C. diaphana* vary considerably in shape, and some Floridian plants match the illustrations given for *C. subdiaphana*. The writer can only conclude that a single species is at hand.

This conclusion is hardened by study of extreme plants of the species (Fig. XVII) from Lake Co., Florida; these are subsequently distinguished as the var. *cristulata*. In these plants the leaves are broadly to narrowly lanceolate, fully as long and narrow as in *C. subdiaphana*; they also show strongly tuberculate-produced cells in the vicinity of the keel, as in the latter species. However, not only the keelar cells are thus armed, but all of the cells of the lobes are

similarly, strongly tuberculate, much as in *C. contractiloba*. These plants are a pure, pellucid green, rather than the usual somewhat opaque yellowish-green of the species. The most distinctive feature of these plants lies in the gemmae: these are usually 16 (rarely 15)-celled, with the marginal cells only 10 in number, of which the usual 3 are gibbous. It is scarcely probable that these plants, on the basis of the fewer-celled gemmae, deserve treatment as a discrete species. An answer to this problem must await study of longer series of specimens, but the plants certainly deserve varietal recognition, as follows:

Cololejeunea diaphana var. **cristulata** var. n.

C. diaphana peraffine. Differt foliis lanceolatioribus; eis sine lobulis modo 4–6 cellulis latis; foliis adultis lobulatis ferentibus magnos inflatos lobulos 0.5–0.65 × longiores lobis; lobus totus et carina argute tuberculata; gemmae recte 16-cellulatae.

Similar to *C. diaphana*, but the lobes ovate-lanceolate to lanceolate, up to 260–275 μ long × 100–110 μ wide, strongly crenulate-denticulate on the margins and along the keel (owing to the strongly tuberculate-produced cells). Lobule up to 150 μ long × 90–100 μ wide, keel arcuate, strongly denticulate. Cells and trigones strongly and irregularly thickened. Gemmae 15–16-celled, or rarely isolated ones 18–20-celled.

TYPE: Alexander Springs, south of Astor Park, Ocala Ntl. Forest, Lake Co., Florida (*R. M. and O. M. Schuster 31510*, Dec. 29, 1953); the type material from a damp to wet log, associated with *Crossotolejeunea bermudiana* and *Riccardia multifida* var.

DISTRIBUTION.—Central and northern Florida, occurring within the range of the species proper.

Florida: LIBERTY Co. (Small twigs, shaded, damp ravine, Alum Bluff on Apalachicola R., *RMS 33470, p.p.*, with *Lejeunea flava;* same locality, on bark of *Illicium floridanum*, *RMS 33529*). MARION Co. (Juniper Springs, on bark of *Quercus nigra*, *RMS 31919;* same locality, on *Vitis* bark, *RMS 31936;* 3–4 mi. below Juniper Springs along Juniper Creek, *RMS 33461*). LAKE Co. (damp to wet log, Alexander Springs, Ocala Ntl. Forest. *R. M. and O. M. Schuster 31510, type;* same locality, on bark of *Acer rubrum*, *RMS 33427;* same locality, on bark of *Azalea serrulata*, *RMS 33429*). HERNANDO Co. (Annuttalagga Hammock, *H. and E. St. John, J. K. Small, Apr. 10, 1936, p.p.*). CLAY Co. (Exposed roots of trees, ravine in Gold Head Branch State Park, *RMS 37203, p.p.*, with *Aphanolejeunea ephemeroides*, *Cololejeunea contractiloba*, *Drepanolejeunea bidens* subsp. *sabaliana*). The Clay County material without mature gemmae, but in part with strongly tuberculate leaves (at least in the keelar region), and with the narrow, lanceolate leaves of the variety.

The variety occurs in very humid and damp sites, either on decaying logs at the edges of shaded springs (*type;* with *Crossotolejeunea bermudiana*, *Riccardia multifida* var.), or on the lower portions of the trunks of trees and shrubs (of *Quercus nigra*, *Azalea serrulata*, *Acer rubrum*, *Illicium floridanum*, etc.). Associated on bark are: *Lejeunea bullata* and *flava*, *Leucolejeunea unciloba*, *Cheilolejeunea rigidula*, *Frullania kunzei*, *donellii*, and *obcordata*, *Diplasiolejeunea rudolphiana*, *Drepanolejeunea* sp. A very few plants are also at hand, growing on the leaves of *Trichomanis petersii*. These plants, from Annuttalagga Hammock, are admixed with a little *Cololejeunea contractiloba* and with *Leptocolea cardiocarpa*. The collection was determined by Dr. A. W. Evans as *C. tuberculata*, but no plants of that species could be located.

Cololejeunea diaphana var. *cristulata* represents the tuberculate extreme of *C. diaphana s. str.*, from which it is normally also distinguished by the fewer-celled gemmae. Typically the gemmae are 16-celled (Fig. XVII: 8) or even 15-celled (as in the type, and in *RMS 33529*). Occasionally, however, individual gemmae are found that are formed of up to 18–20 cells (as, *i.a.* in *RMS 33427*). In var. *cristulata* all of the cells of the lobe, as well as the cells along the keel, are strongly tuberculate-elevated. In typical *C. diaphana* the marginal cells, those along the keel, and occasionally some of the other laminar cells may be weakly tuberculate-produced, but never to the marked extent characteristic of the var. *cristulata*. The strongly roughened cells of the variety are correlated with the presence of generally large trigones and frequent intermediate thickenings.

C. diaphana var. *cristulata* at least occasionally produces gemmae on both the antical and postical surfaces of the lobes (as, for instance, in *RMS 33529*). In this respect, the species recalls the *C. minutissima* complex. Gemmae are usually commonly produced, while sporophytes are rare. However in the plants from Clay County (*RMS 37203*) gemmae are very rare, no mature ones being evident, whereas perianths and capsules are frequent.

The perianth of the var. *cristulata* does not appear to differ to any degree from that of typical *C. diaphana* (or of the evidently synonymous *C. subdiaphana*). It ranges from 220–240 (260) μ wide $\times$ 300–325 μ long, is obovate to obpyriform, and has a smooth surface, except on the distal portions of the keels. It is flat antically, quite lacking the antical keel supposedly characteristic of *Cololejeunea* (and supposedly separating that genus from *Leptocolea*), while the postical keel is broad and two-angled, evidently being formed by the union of the two normally discrete ventral carinae. The lateral carinae are relatively sharp, denticulate with projecting cells, and sometimes angularly produced outward, although hardly to the extent shown by Jovet-Ast (*l.c.*, Fig. VI: 12). The keels are abruptly contracted distally, with the consequence that the perianth is truncate at the apex, if the short beak is excepted. The bracts are long, equalling or even exceeding the mature perianth, with the lobes lanceolate to oblong-lanceolate, occasionally weakly falcate, ranging from 300–370 μ long $\times$ 80–100 μ broad. The lobules are sometimes united for almost their entire length to the lobes (as figured by Jovet-Ast, *l.c.*, Fig. VI: 9, 12), and range from (120) 150–200 μ long $\times$ 40–60 μ broad. More frequently the narrowly oblong lobules are free for $\frac{1}{5}$ to $\frac{1}{4}$ of their length, with the free portion acute to subacutely triangular.

In the specialized perianth, with the antical face devoid of any carina, and with the sharp lateral keels often produced as obtuse to subacute, if rather weak, shoulders, *C. diaphana* and its var. *cristulata* represent the most specialized element among the local species of *Cololejeunea*.

APPENDIX TO *COLOLEJEUNEA MACOUNII* (SPRUCE EX UNDERW.) EVS.

In the preceding article, *C. macounii* was discussed and illustrated. Its evident isolation, taxonomically, from the other North American species was emphasized, while its close affinity to "such east Asiatic species as *Cololejeunea denticulata* (Horikawa) Hattori" was assumed. To confirm this relationship a small fragment of the type of *C. macounii* was submitted to the eminent Japanese

Hepaticologist, Dr. S. Hattori, whose study of the material led him to the following conclusion:

"*Cololejeunea macounii* is identical to the northern material of *Cololejeunea rupicola* Stephani [a species distinctly allied to *C. denticulata*], such as specimens collected in Hokkaido, 42–45° N. Lat., 140–145° E. Long. The description of the latter was given in Vol. 5 of 'Species Hepaticarum' under the genus *Physocolea*. Recently I cited, as its synonym, *Physocolea handelii* Herzog (*in* Mazzetti, Symb. Sinic. V., 1930) and *Physocolea papillosa* Horikawa (*in* Sc. Jour. Hiroshima Univ., Vol. 1, 1932.)" In 1953, Benedix stated in Feddes Repert . . . that Hattori's opinion, "dass *C. handelii* mit *Physocolea rupicola* St. identisch sei, kann ich nicht bestimmen. Allein schon die Blattgestalt ('oblong-elliptisch'), deren Oberfläche ('cuticula levis'!) und das Verhältniss der Zellgrössen (Randzellen!), die Stephani für *Ph. rupicola* angibt, haben mit. *C. handelii* gar nichts zu tun. . . ." However, Dr. Hattori states (*in lit.*) that "Stephani described [*C. rupicola*] as Dr. Benedix points [out]. However, Stephani's description is often incorrect. My decision was based on my examination of the type specimen, deposited in the Herbarium of Kyoto University; U. Faurie's collection made on Mt. Diamond in Korea! Recently I re-examined the type of *Cololejeunea rupicola*, and confirmed the dense papillose verrucae on the antical surface of the leaf and other characteristics which show this species should be conspecific with *Ph. handelii* and *Ph. papillosa*."

On the collective basis of a comparison of three specimens from Japan, of *Cololejeunea rupicola* Steph., kindly sent by Dr. Hattori (Bark of *Pinus densiflora*, Mt. Kirishima, *ca.* 1000 m. alt., Kyushu, *T. Shib 3269;* on living leaves of *Tujopsis dolabrata*, *ca.* 1110 m., Mt. Wanizuka, Miyazaki Pref., Kyushu, Japan, *D. Shimizu;* on bark, *ca.* 800 m. alt., Mt. Hikosan, Fukuoka Pref., Kyushu, *Y. Kuwahara 1279*), which I have compared with the type of *C. macounii*, and on the basis of Dr. Hattori's study of the same materials, and of the type of *C. rupicola*, the following synonymy therefore becomes necessary:

Cololejeunea macounii (Spruce ex Underw.) Evs.

Physocolea rupicola Steph., Spec. Hep. **5**: 904, 1916 (new synonymy).[7]

Physocolea handelii Herzog, *in* Mazzetti, Symbol. Sinic. **5**: 55, fig. 21, 1930 (new synonymy; *teste* S. Hattori).

Physocolea papillosa Horikawa, Bot. Mag. Tokyo **44**: 182, 1932; Jour. Sci. Hiroshima Univ. Ser. B., Div. 2, **2** (2): 287, 1934 (new synonymy; placed as synonym of *C. handelii* by Hattori, Bull. Tokyo Sci. Mus. **11**: 100, 1944).

Cololejeunea rupicola Hattori, Bull. Tokyo Sci. Mus. **11**: 99, 1944; Hattori, Jour. Hattori Bot. Lab. No. 5: 44, 1951 (with *C. handelii* cited as a synonym); Hattori, Jour. Hattori Bot. Lab. No. 8: 37, 1952; Schuster and Hattori, Jour. Hattori Bot. Lab. No. 11: 63, pl. 3, fig. 4, 1954.

Cololejeunea handelii Hattori, Bull. Tokyo Sci. Mus. **11**: 100, 1944.

Cololejeunea macounii, as the species must properly be called, now acquires a characteristic oceanic range, involving both sides of the North Pacific. Parallel cases are those of the species of the genus *Macrodiplophyllum* (Buch) Persson

[7] Dr. Hattori points out that, whereas Stephani gives the type locality as "Formosa," the material actually came from Korea!

(see Persson, 1949; especially Fig. 3). Hattori (1952) cites *C. rupicola* from Formosa, China and Korea, northward to Japan (Honshu, Shikoku, Kyushu, and Yakushima). Unlike *Macrodiplophyllum plicatum*, which extends in a complete arc from Washington to Alaska and to Japan, *Cololejeunea macounii*, which represents a group initially restricted to tropical and subtropical climates, has been unable to extend its postglacial range northward to encompass the central portions of this arc. As a consequence, its existing range appears confined to a rather large oceanic area on the Asiatic side of the North Pacific, while it is of very restricted distribution on the American coast of the North Pacific.

The Japanese material studied demonstrates the correctness of the writer's assumption of an autoecious inflorescence for this species. The previously undescribed androecia appear to occur on abbreviated, leafless, lateral branches, often in the immediate vicinity of the perianth-bearing branches. The male bracts are from somewhat to greatly smaller than vegetative leaves, imbricate and nearly erect, with a long and strongly arched keel, and with subequal lobes (the postical being only slightly shorter than the antical); the 2–3 pairs of bracts form a compact androecium, only some 240–270 μ wide (*vs.* 550–750 μ wide vegetative shoots in the Japanese specimens studied, which are slightly less robust than the American material). The Japanese specimens also diverge very slightly from the American in the form of the 2-celled apical lobular tooth. In the American plants (Fig. XI: 7) the cells of the apical tooth are only slightly elongated, and the hyaline papilla is distinctly ental; in the Japanese material the apical tooth is formed of more elongated cells, the basal cell being quite elongated, and the hyaline papilla is only slightly entally displaced from the "normal" position at the proximal base of the apical tooth.

The oil-bodies of the Japanese plants have been figured and described by Schuster and Hattori (1954, p. 63, Pl. III, fig. 4). They occur 3–7 per marginal cell, 4–10 per median cell, are almost homogeneous (containing minute and indistinct granules); the median ones range from spherical and 2–3 μ to oblong-fusiform and 2–3 $\times$ 3–5 μ. They thus conform closely to the *C. biddlecomiae* type. The gemmae, which were not described in the preceding article in this series, are also similar to those of *C. biddlecomiae* in lacking the gibbous "organs of attachment," although they differ in being formed of only 16 cells (Evans, 1911, Fig. Ic). As a consequence, a rather close relationship between the Sectio Calcareae (to which *C. biddlecomiae* belongs) and the Sectio Denticulatae (to which *C. macounii* belongs) may be assumed.

DIPLASIOLEJEUNEA (SPRUCE) SCHIFFN.

Lejeunia Mont. and Nees, *in* Mont., Ann. Sci. Nat., Ser. 2, **19**: 264, 1843 (in part).
Lejeunia sectio *Duplicatae* G. L. et N., Syn. Hep. 392, 1845 (in part).
Lejeunea subg. *Diplasio-Lejeunea* Spruce, Trans. and Proc. Bot. Soc. Edinburgh **15**: 301, 1884.
Diplasiolejeunea Schiffner, *in* Engler and Prantl, Nat. Pfl.—Fam. **1** (3): 121, 1895; Evans, Bull. Torrey Bot. Club **39**: 209, 1912.

Plants *closely appressed* to substrate (smooth bark; living leaves), forming flat, *small, irregular or radiating patches, pale to yellow-green, rather opaque* (with drying or age occasionally pale brownish), somewhat delicate in texture, *medium sized* (averaging larger than

Leptocolea; closely approaching Radula in size and facies), *mostly 1–1.8 mm. wide* to 2.5–3 cm. long. Stems closely adnate to substrate, irregularly pinnate, *ca.* 85–145 μ in diam.; branching of the *Radula*-type; cortical cells 25–50 μ in diam., subisodiametric in cross-section, *in 7 rows;* medullary cells *ca.* 20–35 μ in diam. *in only 3 rows* (1 for each row of merophytes); cortical and medullary cells both somewhat thick-walled, the cortical not forming a hyalodermis, little distinct from the medullary. Rhizoids frequent, in tufts from bases of underleaves. *Leaves and underleaves arising by means of a zig-zag mode of segmentation*, thus with 1 underleaf for each lateral leaf. Lateral leaves loosely to closely imbricate, large, laterally spreading, *appressed to substrate, flat to slightly convex*. Lobes ovate to suborbicular, *broadly rounded distally*, entire-margined, attached by a very short and nearly transverse line; antical base somewhat ampliate, arching across stem (stem hidden from view in dorsal aspect). Lobule similar in most respects to that of *Cololejeunea*: typically inflated, rather large, *ca.* 0.3–0.6 × the length of the lobe usually, broadened from a very narrow base, obovate to elliptical; keel distinctly arched; free margin involute at least near base, sometimes to above the middle, the distal portions of free margin flatly appressed to lobe; *apical region of lobule bearing two teeth*, the distal (*apical*) tooth various, *prominent*, often acuminate or subulate, sometimes broadened from a stalk-like base and T-shaped; proximal tooth less prominent, different in shape, sometimes obscure or involute; hyaline papilla at base of apical tooth, entally displaced (usually situated near median line of tooth, but often towards distal side of inner face of tooth). *Cells smooth*, at most slightly convex, never conoidally produced; cells usually regularly hexagonal, scarcely or not at all elongated; walls often nearly equally thick-walled, at times with distinct (but not bulging) trigones and isolated, sometimes salient, intermediate thickenings; *cells usually dimorphic*: the majority with several (often 3–7) small, oil-bodies, each formed of few segments, a minority containing a single large, subspherical oil-body; leaves thus with scattered ocelli (*Stictolejeunea*-type). Underleaves *one per lateral leaf, large*, distant to somewhat imbricate, *typically deeply bifid with lanceolate, long, strongly divaricate lobes* which are acute to narrowly obtuse at apex; base of underleaf with well-developed rhizoid-initial disk. *Asexual reproduction by means of discoid gemmae* from the under surface of the lobes (sometimes of unmodified leaves, as in *D. pellucida;* sometimes from modified leaves, as in *D. rudolphiana*); gemmae broadly elliptical in outline, with 3 gibbous "slime cells" (organs of attachment), otherwise with even contours.

Autoecious or dioecious. Androecia compactly spicate, either terminal on short or on leafy branches, rarely proliferating vegetatively; bracts usually in 3–6 (10–12) pairs, *small, densely imbricate*, usually diandrous. Gynoecia on either leading or short lateral branches, invariably *subtended by 1 subfloral innovation*. Bracts much smaller than vegetative leaves, subequally bilobed, the lobule sometimes even slightly longer than the lobe. Bracteole free, more or less bilobed, the lobes varying from widely divergent to suberect, to connivent. *Perianth more or less compressed, but sharply 5-carinate*, obovoid in outline, suddenly narrowed into a short beak distally; keels and intervening surfaces smooth, never tuberculate or dentate.

TYPE: *D. pellucida* (Meissn.) Schiffn. (= *Jungermannia pellucida* Meissner ex Sprengel, *in* Linnaeus, Syst. Veg. Ed. 16, **4**(2): 325, 1827).

The genus *Diplasiolejeunea* is one of the most sharply marked and distinctive of the genera of Lejeuneaceae. It is allied rather distantly to the remarkable genus *Colura*, in which the erect leaves are even more highly derivative, bearing a movable structure allowing the lobules to be closed. *Diplasiolejeunea* shares with *Colura* the very similar underleaves and the 5-keeled perianth. The perianth in the various species of *Colura*, however, is usually provided with horns.

An even more distant relationship may be assumed with *Hemilejeunea* Schiffner (Hedwigia **81**: 37–39, 1942) which differs at once in the linear-lingulate underleaves, which, according to Schiffner, really represent merely the halves of

normal underleaves, each half becoming associated with a lateral leaf and the two halves distinctly separated from each other. The very small and reduced lobule of *Hemilejeunea* is also distinctive. Perhaps nearly as distantly allied is *Calatholejeunea* Goebel (Ann. Jard. Bot. Buitenzorg **39**: 3–8, 1928), which is most closely allied to *Colura*, but bears only slightly emarginate underleaves.

On the other hand, *Diplasiolejeunea* is extremely closely allied to the recently described, monotypic *Aphanotropis* Herzog (Trans. British Bryol. Soc. **2**(1): 63, 1952), from Borneo. This latter genus shows division of the shoot-system into a basal rhizomatous region and distal, leafy portion; only the former bears rhizoids. The lobule of *Aphanotropis* is also reduced to a narrow, explanate fold, much as in normal phases of *Cololejeunea minutissima myriocarpa;* this, conceivably, can be a response to the occurrence on wet rocks along a torrent. *Aphanotropis* also bears conspicuously cymosely oriented inflorescences, and has trigonous perianths. The latter feature strongly suggests that this genus is distinct from *Diplasiolejeunea*.

The distinctive underleaves, present in twice the "normal" number,[8] serve to separate *Diplasiolejeunea* from the other regional genera of Paradoxae, *Aphanolejeunea*, *Cololejeunea* and *Leptocolea*, all of which lack underleaves. However, in spite of this marked difference, the relationship to these genera is clear. In the latter three genera, as well as in *Colura* and *Diplasiolejeunea*, segmentation is of a totally anomalous sort, not proceeding in the usual helical fashion which characterizes other genera of leafy Hepaticae, with a tetrahedral apical cell. Segmentation was shown by Goebel (1898, p. 286) to proceed in a zigzag manner in *Colura*. Evans (1912, p. 209) reports that an examination of the growing points of *Diplasiolejeunea* reveals an identical pattern of segmentation. As a consequence of this zigzag mode of segmentation, every second segment cut off develops into an underleaf, resulting in a 1:1 ratio between leaves and underleaves. No other group of Jungermanniales shows a similar mode of segmentation, or similar ratio between number of underleaves and leaves.

The species of *Diplasiolejeunea* all appear to possess ocelli. When dead plants of considerable age are examined this may not be apparent, since the ocelli are of the "*Stictolejeunea* type," i.e. scattered through the lamina, but never localized in a basal group or vitta. Although Evans (1912, p. 211) stated that ocelli were numerous in *D. rudolphiana* and the other species from Puerto Rico, Frye and Clark (1947) inexplicably state (p. 920, 921) that ocelli are lacking in *D. rudolphiana*. The ocelli and oil-bodies are illustrated and described in detail in Schuster and Hattori (1954).

Diplasiolejeunea is almost exclusively tropical in distribution. Of the 24 species assigned by Stephani to the genus, Evans (1912) reduces one to synonymy, but adds two new species (Evans 1912, 1912a). Jones (1954) has recently transferred an additional African species to the genus, and Herzog has described several

[8] It has been pointed out (Evans, 1912) that duplication of underleaves—hence a pendulum-like mode of segmentation—is not universal in *Diplasiolejeunea*. The bases of branches, as well as the androecia and gynoecia, possess the primitive, spiral mode of segmentation, and show one underleaf for every two lateral leaves (or bracts).

additional new species (*D. patelligera*, 1948; *D. longilobula*, 1950). The total number of species in the genus, therefore, is approximately 28, of which several will surely prove ill-founded. Of these species only a single one occurs in North America. Even this barely penetrates into areas with regular occurrence of frosts. Some of the species have a wide distribution, such as *D. pellucida* (known from tropical America, Africa, the East Indies and New Caledonia). Other species are known from Africa and Tasmania, and from Java; the rest of the species are from tropical and subtropical America. The species occur usually on smooth substrates, usually living leaves or smooth barked trees, on which they form characteristic, pale to yellow-green, flat, closely adnate, radiating small patches, with much the appearance of species of the genus *Radula* or *Leucolejeunea*.

Diplasiolejeunea rudolphiana Steph.

Lejeunea unidenta Mont. *in* Ramon de la Sagra, Hist. Fis. Pol. y Natur. Cuba **9:** 478, pl. 19, fig. 2, 1845 (not of Lehm. & Lindenb., Stirp. Pugil. **6:** 48, 1834).

Diplasiolejeunea unidenta Evans, Bull. Torrey Bot. Club **32:** 183, 1905 (not of Schiffner, Bot. Jahrb. **23:** 583, 1897).

Diplasiolejeunea rudolphiana Stephani, Hedwigia **35:** 79, 1896; Evans, Bull. Torrey Bot. Club **39:** 220, pl. 17, figs. 13–17, 1912; Schuster and Hattori, Jour. Hattori Bot. Lab. No. 11: 60, pl. 13, figs. 1–4, 1954.

Plants in flat, small, opaque, *pale yellow-green patches;* shoots 1.0–1.8 (2.1) mm. wide. Stems *ca.* 100–120 μ in diam., sparingly and irregularly monopodially branched, the branches widely spreading, similar to the main stems (but with somewhat smaller leaves). Leaves clearly imbricate, *somewhat convex*, the lobe widely spreading, closely appressed to substrate (at least by its margins), broadly ovate, 1200–1400 μ long × 1000–1200 μ wide, entire-margined; antical margin ampliate and strongly curved at base, arching across stem, arching outward evenly to the apex; postical margin similarly curved, from the obtusely indented apex of the keel. Lobule oblong-obovate, *ca.* 700 μ long (*ca.* 0.5–0.65 the lobe length) × 350 μ wide, inflated along keel and sometimes nearly throughout, except in the apical region; free margin involute at base, plane and appressed to lobe elsewhere, the *apical tooth long and slender, acuminate mostly 6–12 cells long* × *2 (–3) cells broad* at base, gradually tapering and sharp-pointed, terminating in 2–3 (–5) superimposed subisodiametric cells, lying *virtually parallel to stem* or very slightly spreading; hyaline papilla ental, distad of midline of tooth; *proximal tooth much shorter*, but sharp and distinct, usually terminating in 2–3 superimposed cells, *often inflexed;* keel convexly arched. Cells of leaf-lobe rather equally thick-walled, with slight or no trigones, occasional ill-defined intermediate thickenings; marginal cells *ca.* 15 μ; median cells almost isodiametric and subhexagonal, 20–26 μ up to 22 × 28 μ, at base slightly elongate, *ca.* 25 × 37 μ; chlorophyllose cells with large chloroplasts (*ca.* 4.5 μ) and 3–6 (8) oil-bodies; oil-bodies varying from 1.8–2.5 μ and spherical to sublinear or ellipsoidal and 1.5–2 × 4.8–6.5 μ, occasionally to 2.5 × 7.5–9 μ, or linear and 2.5–3 × 9–12 μ, the smallest homogeneous or of 2–3 segments, the larger of 1–2 rows of coarse segments (segments *ca.* 0.7–1.2 μ). Ocelli nearly or quite devoid of chloroplasts, each containing a single spherical to broadly ovoid oil-body varying from 16–20 μ to 18 × 24 μ. Underleaves large, distant to subimbricate, broadly cuneate in outline, 170–250 μ long medially × 400–600 μ wide apically, bilobed to middle with obtuse to narrowly rounded sinus and *divaricate, obliquely spreading lobes;* lobes narrowly rounded to obtuse at apex, sometimes subacute. Asexual reproduction by gemmae of the undersurface of the leaf-lobes. Gemmae production limited to distal leaves of short, modified branches, definitive in growth; gemmae discoid, with 3 swollen rhizoid-initials.

Autoecious. Androecia always forming short lateral branches, compact, not proliferating

apically; bracts usually in 3–6 (9) pairs, closely imbricate, strongly inflated, subequally bilobed, the lobes obtuse to rounded; bracteoles smaller than underleaves, less deeply bilobed, with oblique, acute to subacute lobes. Gynoecia on elongated or very abbreviated lateral branches, the single subfloral innovation generally short, sterile, or almost immediately fertile. Bracts strongly complicate, the short keel narrowly winged, bifid 0.65–0.75; lobe oblong-obovate; *ca.* 400–500 × 165–200 μ wide, rounded at apex, obscurely crenulate on margins from projecting cells; lobule oblong, 350 μ × 110–120 μ wide, obtuse and emarginate to subacute. Bracteole ovate, *ca.* 350–420 μ long × 210–250 μ wide, 0.2–0.35 bilobed with the short lobes acute to obtuse, erect or suberect; sinus acute. Perianth slightly compressed, sharply 5-carinate, oblong-obovate in outline, *ca.* 475–500 × 675–1300 μ. Capsule shortly pedicellate, *ca.* 300–325 μ in diameter, greenish-brown. Seta of 12 epidermal cell rows, surrounding 4 interior cell rows; cell-walls delicate and pellucid. Epidermal cells of capsule more or less diamond-shaped, the marginal ones *ca.* 20–24 μ, measured along the margins, the interior cells gradually becoming larger and 20–25 × 30–36 μ (a few to 35 × 50 μ), their walls irregular and sinuous, the angles rather strongly nodular-thickened, and the longer walls often each with a coarse nodular thickening. Inner cell-layer much more delicate, inconspicuous, with delicate, inconspicuous, hyaline, nodular thickenings (at least locally). Elaters few, slender, up to 250–295 μ in length × 13–15 μ in diam. above the middle, obsoletely unispiral. Spores green, with a brownish exine marked with numerous small papillae, and a series of coarser papillae arranged in roughly circular patterns, 20–25 (30) μ in diam. × *ca.* 50–65 μ in length before dispersal.

TYPE: Brazil (*Rudolph*); portions of the type at the Herbier Boissier, and at the British Museum.

DISTRIBUTION.—*D. rudolphiana* "appears to be the dominant representative of the genus in the lowlands of tropical America." (Evans, 1912, p. 221). It occurs from Petropolis, Brazil (*type*), northward to Paramaribo, Netherlands Guiana; Panama (*fide* Stephani); British Honduras (Fulford, 1938); and various stations in the West Indies as follows: near Cayey, Puerto Rico; Troy, Jamaica; near Port Margot, Haiti; Cuba; New Providence, Bahama Isls. (Evans, 1912; Pagan, 1939). In our area first reported from Florida by Evans (1905; as *D. pellucida*), and now known from a series of stations in southern and central Florida. Until recently (Redfearn, 1952, p. 196) recorded only from a single collection from Dade County, and one from Seminole County.

The range of the species in Florida at first appears peculiar: it centers about a southern area embracing Monroe, Dade and Collier Counties, and an area in central Florida, including Hillsborough, Seminole, Lake and Marion Counties. The latter stations for the species all lie in or at the periphery of the "Oligocene Island" region. Parallel cases in distribution are quite frequent among the ferns; Small (1938) called attention to a number of them including *Tectaria heracleifolia*, *Goniopteris reptans*, *Asplenium abscissum*, *Polypodium plumula* and *pectinatum*. The explanation advanced by Small to account for this seeming anomaly in distribution also appears to be satisfactory in explaining the distribution of *Diplasiolejeunea:* species with a distribution of this type have entered Florida in two "waves," an old migration during the period before the Tertiary, and a recent, post-Pleistocene or Pleistocene migration. The first wave accounts for the distribution of these species in central Florida; the second, for their presence in the southern tip of the peninsula. Among the Hepaticae, *D. rudolphiana* appears to be the only one known to date with such a bicentric distribution in Florida.

Florida: MONROE Co. (Everglades, along Route 94, 3 mi. s. of Collier County line, *R. M. and O. M. Schuster 31741a, p.p., 31759b, 31753, 31748a*). COLLIER Co. (Collier-Seminole State Park, *RMS 26158b, 20263b*). DADE Co. (near Camp Longview, *Small and Wilson 2058* p.p.! NYBG; Paradise Key, Everglades Ntl. Park, *RMS 33350c p.p., 19930, 20184b, 22740d;* Hammock 5–6 mi. w. of Florida City, on Loveland Road, *RMS 22651a p.p., 22746a, 22760, 22677b;* Dense hammock in Section 24, Long Key pineland, Everglade Keys, Everglades Ntl. Park, *RMS 22053;* Timms Hammock, Bauer Drive, n. of Modello, *RMS 26001*). HILLSBOROUGH Co. (Hillsborough R. State Park, along Hillsborough R., *R. M. and O. M. Schuster 33908a, 33909d, 33944a*). LAKE Co. (Alexander Springs, s. of Astor Park, Ocala Ntl. Forest, *RMS 33316a, 33420, 33425a, 33301, 33436a, 33385*). SEMINOLE Co. (Robinson's Springs, s. of Sanford, *Rapp 68!*). MARION Co. (Along Juniper Creek, 3–4 mi. below Juniper Springs, Ocala Ntl. Forest, *RMS 33456, 33397a;* northernmost station for the genus!). HIGHLANDS Co. (Highlands Hammock State Park, w. of Sebring, *Redfearn 873–68–55*).

ECOLOGY.—*D. rudolphiana* appears to be a very tolerant pioneer species. In our area it has been previously reported only from bark, but it also occurs rather rarely on the leaves of various plants. In central Florida sometimes on the leaves of *Ilex cassine*, associated with *Leptocolea cardiocarpa* (Alexander Springs, *RMS 33420*) but evidently more commonly on the leaves of several species of palms. On the saw palmetto (*Serenoa repens*) in open forest along the Hillsborough R. it may occur with *Cololejeunea minutissima, Leptocolea cardiocarpa,* as well as with traces of *Frullania obcordata, F. kunzei, Lejeunea flava, L. bullata, L. longifissa, Leucolejeunea conchifolia* and *Aphanolejeunea evansii* (*RMS 33908, 33909*). On *Sabal palmetto* it may occur with *Leptocolea, Leucolejeunea conchifolia, Lejeunea laetevirens* and *Cololejeunea minutissima* (*RMS 33944a*), or with *Leptocolea, Caudalejeunea lehmanniana, Aphanolejeunea evansii, Cololejeunea subcristata, Leptolejeunea elliptica* and the moss, *Taxithelium planum* (in *RMS 33850*). Unlike *Leptocolea* and *Leptolejeunea*, which may invade any portion of the palm frond, *Diplasiolejeunea* almost invariably undergoes ecesis only in the pleated basal portions of the frond.

D. rudolphiana occurs more frequently on bark of a wide range of "host" species, ranging from *Quercus virginiana* and *Alvaradoa amorphoides* at the open, relatively sunny edges of hammock forests (Timms Hammock), while at the rather open but humid margins of hammock forests around large springs (Alexander and Juniper Springs) occurring on the bark of *Ilex cassine, Myrica cerifera,* or such shrubby plants as *Azalea serrulata, Leucothöe* cf. *acuminata,* and *Baccharis halimifolia.* Under such conditions it is associated chiefly with *Frullania donellii, F. obcordata, F. kunzei,* occasionally *F. squarrosa* and *F. cobrensis, Cheilolejeunea polyantha, Lejeunea bullata* and *flava, Leucolejeunea unciloba, conchifolia* and *xanthocarpa* and *Cololejeunea minutissima.* In the southern tip of Florida the species is also a "hammock species", found on the bark of *Erythrina herbacea, Roystonea regia, Ficus aurea,* and *Xanthoxylum fagara,* occasionally on *Elapharium simaruba, Dipholis salicifolia, Magnolia virginiana, Ocotea catesbyana,* and other trees. Associated here are *Frullania riojaneirensis, F. squarrosa, F. donellii, Lejeunea bullata, Cololejeunea minutissima, Leptolejeunea, Cheilolejeunea polyantha,* occasionally *Brachiolejeunea bahamensis, Lejeunea flava* and *laetevirens, Caudalejeunea lehmanniana* and *Cheilolejeunea decidua. D. rudolphiana* is also occasionally found on the secondary vegetation along the canals in the Everglades, on *Chryso-*

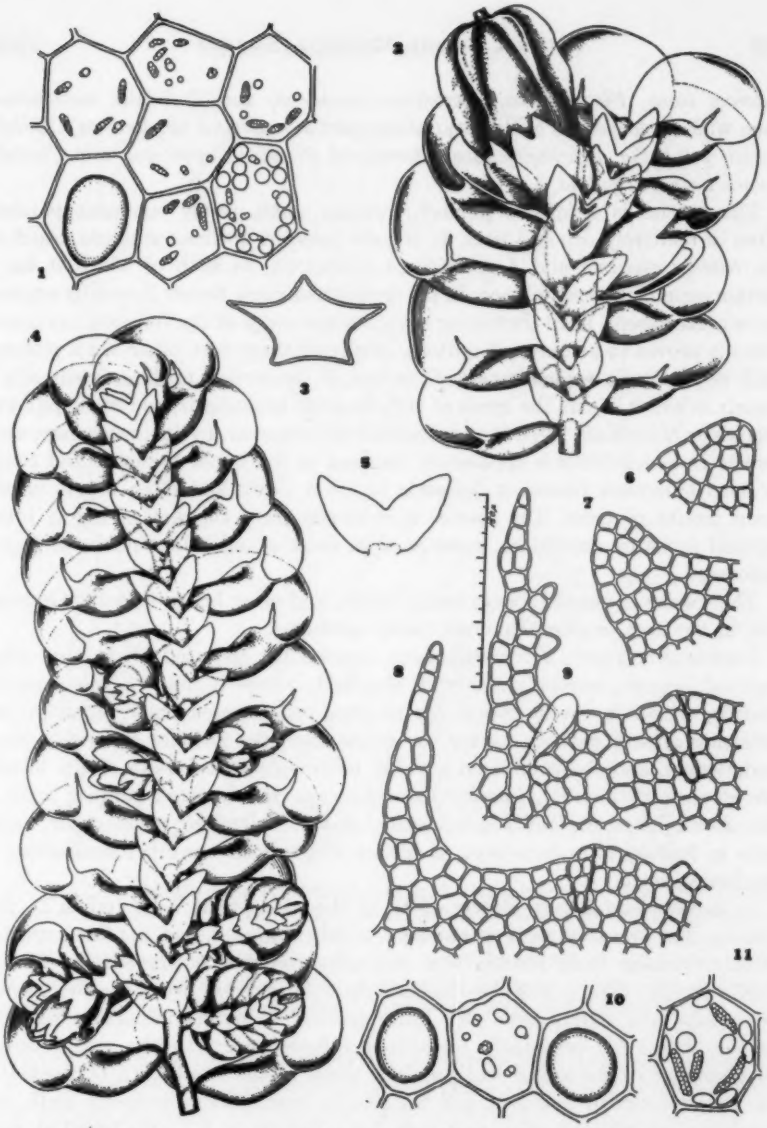

FIG. XVIII. *Diplasiolejeunea rudolphiana* Steph. 1. Median cells, the chloroplasts drawn in only one cell (×640); 2. Lateral branch, with unfertilized gynoecium and perianth (×42); 3. Cross section of perianth (×40); 4. Apex of leading shoot, with one androecial and two gynoecial branches on left side, two androecial and one gynoecial branches on right side; lowest gynoecial branch with two gynoecia (×24); 5. Underleaf (×42); 6–7. Lobes of underleaf in Fig. 5 (×193); 8–9. Apical and preapical teeth of lobules, antical (ental) view, in Fig. 9 the slime papillae drawn in (×193); 10. Three cells, two with ocelli (×640); 11. Cells with chloroplasts and oil-bodies (×640). (Figs. 1, 10–11, Florida, *Schuster 22053* and *26001*, from Schuster and Hattori, 1954; Figs. 2–3, from *Schuster 33425a*, Florida; Figs. 4–9 from *Schuster 31753*, Monroe Co., Fla.).

balanus icaco, *Ficus aurea*, *Taxodium ascendens*, and *Fraxinus caroliniana*; then with *Rectolejeunea brittoniae*, *Cololejeunea minutissima myriocarpa*, *Cheilolejeunea polyantha*, *Brachiolejeunea bahamensis*, *Frullania squarrosa*, and *Cheilolejeunea* near *C. rigidula*.

The species is strictly a pioneer, forming small, rarely confluent patches, often in relatively exposed sites. It is quite tolerant of direct sunlight, much as are *Radula andicola* and *Leucolejeunea conchifolia*, to both of which it has a certain superficial resemblance. In the deeper hammock forests it usually appears to be quite absent, until the higher branches and twigs of the trees are examined, when it proves to be quite ubiquitous, occurring there with other strict pioneers such as *Frullania riojaneirensis*, *F. kunzei*, *F. squarrosa*, forming eventually a matrix in which occurs the ecesis of various small bromeliads, and such epiphytic orchids as *Harrisiella porrecta* and *Epidendrum tampense*. In the deeper hammock forests *D. rudolphiana* is apparently confined to the upper branches and twigs; in open hammock forests it descends to lower levels and often occurs on the main trunks of trees. The species is evidently very tolerant of highly intermittent moisture conditions, more so than most of our other species of Lejeuneaceae.

The species is almost always freely fertile, and often bears capsules. Gemmae are, in our area, apparently quite rarely produced.

DIFFERENTIATION.—*D. rudolphiana* cannot be confused with any other regional hepatic, except possibly in the field, where it may be mistaken for *Radula*, which it closely resembles in size, yellow-green color, opacity, and prostrate growth pattern. Under the microscope, the peculiar divaricate-lobed underleaves, and the prominent slender, acuminate apical teeth of the lobules are at once distinctive. Equally distinctive are the scattered laminar ocelli of the dorsal lobes. In our Lejeuneaceae, scattered laminar ocelli occur, again only in *Rectolejeunea berteroana*, a species which bears no other similarities to *Diplasiolejeunea*.

D. rudolphiana is very closely allied to the widespread West Indian *D. unidentata*. The two taxa were at one time widely confused, and the first report of *Diplasiolejeunea* from Florida was originally referred to this species (Evans, 1905). Evans (1912, p. 219) has outlined the entangled history of the two species. *D. rudolphiana* differs from this latter species in several important respects, among them: (a) constantly autoecious inflorescence; (b) the extraordinary development of the apical and proximal teeth of the lobules. In *D. unidentata* the inflorescence is dioecious, and the lobules bear obscure preapical teeth, and much less prominent apical teeth only 3–4 cells long × 1–2 cells broad at base. The apical teeth in *D. unidentata* also spread obliquely, whereas they are virtually parallel to the axis in *D. rudolphiana*, at least in mature, well-developed individuals.

Although these distinctions appear at first to be sharp and absolute, variation in *D. rudolphiana* serves nearly to bridge the gap between the two species. At least in the Floridian plants, the preapical tooth may be virtually obsolete (i.e. in *Schuster 31748a*, Monroe Co., and *Redfearn 873–68–55*). In such cases

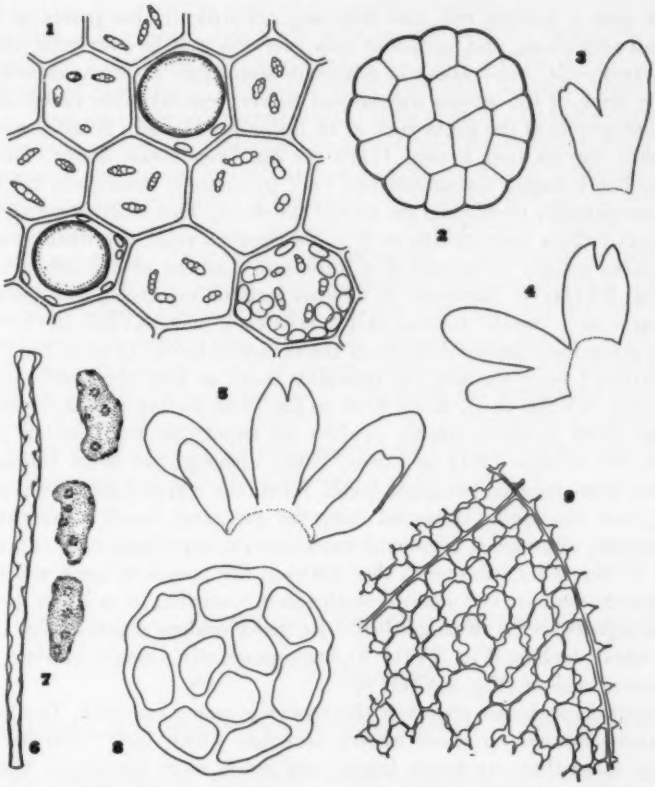

FIG. XIX. *Diplasiolejeunea rudolphiana* Steph. 1. Cells, middle of lobe, with 2 ocelli; chloroplasts drawn in in one cell (×655); 2. Cross-section of seta (×210); 3. Perichaetial bract (×50); 4. Bract and bracteole (×50); 5. Bracts and bracteole (×50); 6. Elater (×210); 7. Spores (×210); 8. Cross-section of stem (×210); 9. Epidermal cells of apical half of valve, with portions of marginal cells from adjacent valves drawn in (×210). (Fig. 1, Timm's Hammock, Dade Co., Fla., *Schuster 26001;* Figs. 2–9, *Schuster 33425a*, Florida).

the apical tooth may also be somewhat less prominent than is typically the case in *D. rudolphiana*, again suggesting intergradation to *D. unidentata*. However, without exception, all Floridian collections are clearly autoecious.

Another close relative of *D. rudolphiana* is another West Indian species, *D. johnsoni* Evans (see Evans, 1912a, p. 603). This latter species agrees with *D. rudolphiana* and *D. pellucida* in the obtuse to rounded, broad lobes of the underleaves. It furthermore agrees with *D. rudolphiana* in the autoecious inflorescences. However, *D. johnsoni* is at once separable from *D. rudolphiana* on the basis of (a) the more convex dorsal lobes, with revolute margin, the margin in the vicinity of the apex of the keel being so strongly revolute that the base of the sinus is displaced; (b) the apical tooth is short, relatively blunt, rarely tipped

by more than a solitary cell, and obliquely oriented; (c) the preapical tooth is blunt and very small, and formed of only two cells that lie over each other.

VARIATION.—*D. rudolphiana* is relatively stenotypic in most respects. However, the teeth of the lobules are subject to very considerable variation, much as in other species of the genus such as *D. pellucida* (Meissn.) Schiffn., a common neotropical species (see Evans, 1912). As has been noted above, the species typically has a highly elaborated and very prominent, acuminate apical tooth which lies virtually parallel to the axis of the shoot. This tooth is usually entire, and tapers from a basal width of 2, more rarely 3 cells; the distal one-fourth to two-fifths usually is formed of a uniseriate filament of 3–5 subisodiametric cells (Fig. XVIII: 8). However, in at least isolated instances, this apical tooth may bear 1 or 2 "teeth" formed of 1–2 cells each (Fig. XVIII: 9). Even more variable is the form and orientation of the preapical tooth. In all of the Floridian plants which I have studied, the preapical tooth at first appears to be wholly absent (Fig. XVIII: 2, 4), in contrast to the West Indian plants, in which the preapical tooth is often readily evident on superficial examination (see, for instance, Pl. 17, figs. 13–14 in Evans, 1912), although the West Indian plants also often show inflexed preapical teeth. When the regional plants are carefully studied, and the lobule dissected free, the preapical tooth is almost aways demonstrable, although it is inflexed and hidden in superficial view (Fig. XVIII: 4, 8–9). Evans (1912) also notes that between the preapical tooth and the base of the lobule "one or two minute teeth, each consisting of a single projecting cell, can sometimes be distinguished." In the Floridian plants these teeth are usually wholly lacking (Fig. XVIII: 9), but occasionally a single, obscure, 1-celled projection is evident (Fig. XVIII: 8).

The position of the sex organs in this species is rather constant. The androecia are always situated on short lateral branches which lack "normal" leaves. In many cases they are much longer and much more prominent than those illustrated (Fig. XVIII: 4), forming compact, elongated spikes of as much as 8–10 pairs of bracts, much as is sometimes the case in *Leptocolea cardiocarpa*. The gynoecia, by contrast, always appear to occur on lateral branches of more or less reduced size. Often (Fig. XVIII: 4) the gynoecial branch is extremely short, devoid of "normal" leaves, and soon is floriferous. In other instances (Fig. XVIII: 2), the gynoecial branch is relatively elongated and bears 3–6 or more pairs of vegetative leaves that are normally developed, although appreciably smaller than those of sterile axes. In either case, a single innovation is developed almost immediately, which is evident at the time of maturation of the single archegonium. This innovation, in the cases studied by the writer, is almost immediately again fertile (Fig. XVIII: 2, and lower gynoecial branch in Fig. XVIII: 4), although the innovation produced at the base of the second gynoecium is apparently usually short and sterile. Evans (1912, p. 220) described his material as most often with short and sterile innovations, a condition which is exceptional in the Floridian plants which have been examined. The bracts and bracteoles of the Floridian plants agree almost perfectly with those described and illustrated by Evans.

In addition to the preceding level of variability, a series of recently collected specimens from a Mahogany-Royal Palm hammock in the Everglades (southwest of the tip of Long Pine Key, west Dade Co., *RMS 37328a, 37332b, 37333a, 37339a, 37340, 37313a, 37310, 37306c, 37331f*) have been studied. These plants occurred in the open hammock margin on a variety of trees (*Rapanea guianensis, Eugenia axillaris, Swietenia mahogani, Ilex cassine, Chrysobalanus icaco, Magnolia virginiana*) and deeper in the hammock on Royal Palm (*Roystonea regia*), associated with a large variety of other hepatics (*Frullania donellii, F. obcordata, Lejeunea bullata, Leucolejeunea xanthocarpa, Leptolejeunea elliptica, Cheilolejeunea cf. polyantha, Leptocolea, Caudalejeunea, Cololejeunea minutissima myriocarpa, Harpalejeunea stricta, Brachiolejeunea bahamensis, etc.*). Some of these collections were wholly typical, but in some cases (*i.a.*, in *RMS 37310*), the plants were totally deviant in that the apical tooth of the lobule was very highly elaborated, bearing not only one to several teeth, but sometimes even well-developed cilia or lobes. In such cases, the apical tooth had a dendroid or ramified form, wholly distinct from the normal form. Since intergradation occurred between this elaborated form of lobular tooth and the "normal" type, it is felt that taxonomic recognition of this variant would be premature.

RARE OR UNUSUAL SLIME MOLDS FROM NORTH CAROLINA[1]

BY T. W. JOHNSON, JR.

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There are apparently no published accounts dealing specifically with the slime molds of North Carolina. In two unpublished theses, Doubles (1938, 1940) lists 20 and 41 species, respectively, collected in the state, six of which were designated as first records. Welden (1951) reported 83 species from the Smoky Mountain region of eastern Tennessee and western North Carolina, but only a few were collected in the latter state. The writer has collected 146 species, either as mature fructifications in the field or by bark culture technique. All of the species reported by Doubles and Welden have been found in the state. The present paper is a report on some unusual species, the majority of which have not heretofore been collected in North Carolina.

In the interest of brevity, the records are cited by counties only. The nomenclature accepted by Martin (1949) is used. Dr. I. Mackenzie Lamb very kindly furnished, from the Farlow Herbarium, authentically determined specimens for comparative purposes.

[1] Funds from the Duke University Council on Research, for travel connected with the study of slime molds of North Carolina, are gratefully acknowledged.

LICEALES

Licea biforis Morgan. Chatham and Orange Counties. Developed in moist chambers on white oak bark. The dark yellow-brown, sessile fructifications which dehisce by means of a longitudinal fissure, suggest a minute hysteroid fungus. The sporangia are small, the largest measuring 0.4 mm. in length. In the Carolina collections, the spores are smooth; these are minutely roughened, according to Martin (1949).

Licea operculata (Wingate) Martin. Catawba County. Developed in moist chamber on dogwood bark. The small sporangia, concolorous with the substratum, are easily overlooked. The species is recognized by the thin, membraneous, preformed operculum, which, in the Carolina collection, is concolorous with the black peridium, and not yellow as reported in previous descriptions.

Cribraria minutissima Schweinitz. McDowell County. A rather generous development of a pallid form of this minute species appeared on southern red oak bark in a moist chamber. The peridial net suggests a glass-like halo around the central spherical mass of pale yellow spores. The net is without nodes, and consists merely of flattened, colorless threads which are only very slightly expanded at their junctures. The included colorless dictydine granules give an impression of poroid threads. Expanded nodes are present in "typical" *C. minutissima*, and the net is yellow-brown or orange-brown.

TRICHIALES

Dianema corticatum Lister. Cherokee County. Only one station is known for this species, which has a recorded southern range in Pennsylvania. The plasmodiocarps in the single scanty development are small and annulate. The capillitium consists of scanty, simple, variously-marked threads. The clusters of spores, echinulate on the exposed surfaces, are characteristic for this species.

Prototrichia metallica (Berkeley) Massee. Caldwell and Burke Counties. This species was collected in the Great Smoky Mountain National Park by Hagelstein (Linder, 1941), and is sufficiently rare to warrant a second report. The fascicled or penicillate arrangement of the capillitial threads is diagnostic for the species, as is the iridescent yellow or orange peridium.

Arcyria oerstedtii Rostafinski. Rowan, Davidson, and Durham Counties. The principal diagnostic feature is the presence of peridial patches on the mature, expanded capillitium. The species has a distinctly echinulate capillitium with no suggestion (in the Carolina material, at least) of warts, cogs, or rings as capillitial markings. Sporangial coloration is suggestive of brownish forms of *A. incarnata*, but the spiny capillitium is diagnostic.

Hemitrichia intorta (Lister) Lister. Northampton County. A single development consisting of a few sessile sporangia. The slime mold has been reported as far south as Pennsylvania. Superficially, the species suggests a trichia by reason of the apparent free ends of the capillitium. The free ends are in reality long, twisted elaters projecting from the capillitial mass.

STEMONITALES

Diachea thomasii Rex. Jackson County. The species is known only from North Carolina, Pennsylvania, and Tennessee (Martin, 1949: 72). It is easily distinguished from others in the genus by the bright orange or deep-yellow stipe, and the conspicuous orange hypothallus.

Enerthenema berkeleyanum Rostafinski. Guilford County. Previously reported by the writer (1955).

Stemonitis trechispora (Torrend) Macbride. Richmond County. Virginia has been reported as the southernmost range for this species. The sporangia are predominantly sessile, but some short-stipitate fruitings occur. The main capillitial threads arising from near the base of the columella are branched principally at their apices. Near the center and apex of the columella the capillitial threads are tenuous, and branch repeatedly near their point of origin from the columella. Quite commonly the columella is very stout in the basal portion of the sporangium, often half the diameter of the fructification.

Stemonitis webberi Rex. Durham County. This is chiefly a western species, which may be easily confused with *S. splendens*. The very large surface net meshes of *S. webberi* usually serve as a dependable diagnostic characteristic. Although the sporangia of this species are usually considerably shorter than those of *S. splendens*, the size ranges of these two species cannot be used confidently in identification.

Stemonitis carolinensis Macbride. Wake County. This species may be mistaken for *S. pallida*, but the small-meshed surface net, the meshes often smaller than the diameter of the spores, is distinctive.

Comatricha irregularis Rex. Craven County. The capillitium is the distinctive feature, in fully blown sporangia forming a distinct but irregular inner net. At the periphery of the sporangium, the net is imperfectly formed, and numerous free ends are observable. The sinuous columella reaches the sporangial apex.

Comatricha suksdorfii Ellis and Everhart. Rockingham County. Principally a western species, although reported by Welden (1951) in Tennessee. The species is distinct because of its very dense capillitium, the individual strands of which are very dark brown or black. Hence, the macroscopic aspect of the fructification is sooty or black.

Comatricha fimbriata Lister and Cran. Durham, Orange, and Jackson Counties. Obtained by moist chamber culture of red cedar bark. Easily recognized by its peculiar capillitial branches the basal portions of which are slender, but with flattened and expanded apices. The species has been found in Florida and Michigan by Alexopoulos (1953), and in Illinois by Benjamin and Poitras (1950). Martin records the species only from Massachusetts, New York, Iowa, and Kansas.

Comatricha cornea Lister and Cran. Buncomb County. Three sporangia developed on atlantic white cedar bark in moist chambers. Martin (1949) reports the species from Iowa and Kansas, Benjamin and Poitras (1950) from Illinois.

Alexopoulos collected *C. cornea* in Michigan (1952) and Florida (1953). Widely distributed, but apparently not unusually rare. The dark-brown stalks fade to yellow-brown near the conspicuous discoid hypothallus. The outer portion of the stalk is solid, and not fibrillose as in *C. fimbriata*. The capillitium is scanty and generally arises from 2–3 major branches from the apex of the short columella. A small "collar" marks the position of the lower portion of the otherwise fugacious peridium. The spores appear gray by transmitted light, and a portion of the wall of each spore is thin.

Clastoderma debaryanum Blytt. Lincoln, Macon, and Orange Counties. The small size of this species may account for its supposed rarity. A fusiform or annulate swelling about midway up the slender, rugulose stipe is a distinctive characteristic. In mature sporangia the apices of most capillitial branches are attached to small peridial patches.

PHYSARALES

Badhamia utricularis (Bulliard) Berkeley. Person County. Although this species may have both sessile and stipitate sporangia, the Carolina collection consists only of the latter. The stipes are thin, pallid or yellowish, and, in most fructifications, little more than tenuous strands of dry protoplasm.

Badhamia foliicola Lister. Durham and Orange Counties. The distinctive characteristic seems to be that of the wrinkled peridium. Macroscopically, the sporangia are blue-gray and iridescent. The spores are characteristically free, but with a tendency to adhere in loose clusters, and are minutely spinulose over their entire surface.

Physarum flavicomum Berkeley. Transylvania County. Developed in a moist chamber on southern red oak bark. Determined by Dr. G. W. Martin. The single development is hardly recognizable as *Physarum* since the capillitium is very scanty and lacks calcareous nodules. The limeless, iridescent, sooty peridium, the lenticular sporangia, and the yellow-brown stipes, however, mark the species. The very sparse capillitium (entirely absent in some sporangia) may be due to moist chamber development, and certainly is far from "typical."

Physarum roseum Berkeley and Broome. Orange County. Prior to this collection, known only as far north as Florida. The stipes are much shorter than described by Martin (1949). The bright purplish-red peridium and the large, dark purple-red lime nodules are distinctive.

Craterium paraguayense (Spegazzini) G. Lister. Transylvania County. Distinguishable from other species in the genus by the bright red, dark purple-red, or light reddish-purple peridium. Most of the sporangia are cyathiform, and reach a maximum height of 1 mm. The calcareous nodules are dark violet, irregular, and connected by pale lilac, non-calcareous threads. The pseudocolumella is often lacking.

Craterium concinnum Rex. Rockingham and Warren Counties. Expanded, funnel-shaped sporangia opening by a preformed, convex lid, mark this very characteristic species. The calcareous nodules are small and dark brown; the peridium is pale brown with a very faint pinkish cast.

Diderma trevelyani (Greville) Fries. Stokes County. A three-layered peridium, the middle one composed of closely aggregated calcareous granules, is characteristic. At maturity the peridium dehisces in a petaloid fashion. In some sporangia the capillitial strands are densely beaded; in others, these are smooth.

Diderma ochraceum Hoffman. Jackson County. Determined by Dr. G. W. Martin. The majority of the sporangia are bicolored, a portion of the calcareous deposits being chocolate-brown, the remainder dull flesh-colored or pinkish-tan. A few sporangia are entirely chocolate-brown. The columella is well-developed. The spores are dark purple-brown; the usual spore coloration is pale purple-gray.

LITERATURE CITED

ALEXOPOULOS, C. J. 1952. New records of Myxomycetes from Michigan. Pap. Mich. Acad. Sci. Arts., Ltrs. **38**: 3–7. (1953).

———. 1953. Myxomycetes developed in moist chamber culture on bark from living Florida trees; with notes on an undescribed species of *Comatricha*. Quart. Jour. Fla. Acad. Sci. **16**: 254–262.

BENJAMIN, R. K. AND A. W. POITRAS. 1950. An addition to the Myxomycete genus *Comatricha*. Mycologia **42**: 514–518.

DOUBLES, J. A., JR. 1938. Myxomycetes of the Chapel Hill area. 25 pp. (Unpubl. M. A. Thesis).

———. 1940. A taxonomic study of the Myxomycetes of North Carolina. 49 pp. (Unpubl. Ph.D. Dissertation).

JOHNSON, T. W., JR. 1955. *Enerthenema berkeleyanum*. Mycologia **47**: 608–611.

LINDER, D. H. 1941. Mycological Society of America: report on the 1939 Foray. Mycologia **33**: 570–578.

MARTIN, G. W. 1949. Myomycetes. North Amer. Flora **1**(1): 1–190.

WELDEN, A. L. 1951. A taxonomic survey of Myxomycetes of the Great Smoky Mountains National Park. Jour. Tenn. Acad. Sci. **26**: 271–276.

SOME WATER MOLDS FROM PUERTO RICO[1]

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There have been few studies dealing specifically with the aquatic fungi of Puerto Rico. Coker and Matthews (1937) for example, do not list a single species of Saprolegniaceae from the island. Chytridiaceous fungi, however, are reasonably well-known. Among the Blastocladiales, representatives of which are reported in this paper, *Allomyces moniliformis* has been collected on the island. According to Sparrow (1943), the record is questionable. This paper presents the results of a study of some filamentous aquatic Phycomycetes occurring in soils along the northern coast of Puerto Rico. Fifteen species were isolated. Two new species of *Brevilegnia*, and a new variety of *B. megasperma* were recovered

[1] Portion of a thesis submitted for the degree of Master of Arts, Duke University, Durham, North Carolina.

from soil. In addition, a parasite of *Allomyces arbusculus*, and several species of *Pythium* were collected. Members of the latter genus were not considered in this investigation.

All fungi reported herein, with the exception of *Rozella allomycis*, were propagated as single-spore isolates, on hempseed halves, in 30 ml. of charcoal-filtered, distilled water. Cultures were incubated at 23–25°C., and characterized when they were 2–2½ weeks old. The new entities were compared with preserved or living material (of isolates propagated under the foregoing conditions) of *Brevilegnia linearis*, *B. unisperma* var. *delica*, *B. unisperma* var. *litoralis*, *B. diclina*, and *B. megasperma*, and with preserved type specimens of *B. longicaulis*.

Brevilegnia crassa n. sp. (Figs. 1–3)

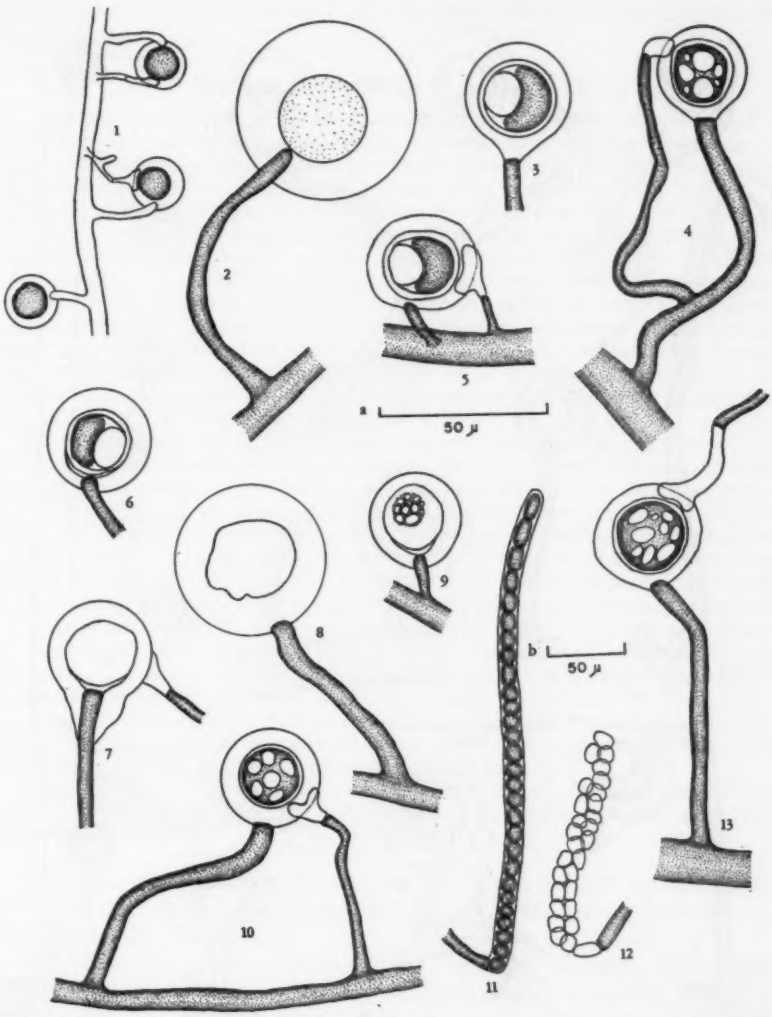
Myceliis densis; nullis gemmis. Zoosporangiis copiosis; fusiformibus vel filiformibus, sympodialiter renovatis; semper 1–2 ordinibus zoosporarum. Zoosporarum emissione brevilegnioida. Oogoniis copiosis, lateralibus, interdum intercalaribus, raro terminalibus; sphaericis vel pyriformibus, nonnumquam subglobosis vel irregularibus; 21–43 μ, plerumque 24–26 μ in diam.; tunicas leves, hyalinas, non-punctulatas, saepe irregulares habentibus; 3.2–17.6 μ, plerumque 4.8–6.4 μ crassis in oogoniis maturis; plerumque 11.2 μ crassis in oogoniis abortivis. Oogoniorum ramulis crassis, nonnullis longis et tenuibus; rectis, nonnullis curvis. Oogoniis immaturis nonnumquam proliferentibus. Oosphaeris aut maturantibus aut non maturantibus. Oosporis sphaericis, quae singulae singulis in oogoniis ita gignuntur ut oogonia omnino impleant; excentricis, nonnullis irregularibus; 11–24 μ, plerumque 19–21 μ in diam. Antheridiis saepissime monoclinibus, interdum diclinibus aut androgenibus. Antheridiorum cellis lateraliter aut apice affixis.

Mycelium dense; principal hyphae slender. Gemmae lacking. Zoosporangia abundant; fusiform or filiform; renewed sympodially; predominantly 216–266 × 8–18 μ; always with 1–2 rows of zoospores. Zoospore discharge brevilegnioid. Oögonia abundant; lateral, occasionally intercalary, infrequently terminal; spherical or pyriform, occasionally subglobose or irregular; 21–43 μ in diam.; predominantly 24–26 μ. Oögonial wall smooth; unpitted; 3.2–17.6 μ thick, generally 4.8–6.4 μ. Oögonial stalks stout; generally twice the diameter of the oögonium in length; straight, occasionally bent. Immature oögonia occasionally proliferating. Oöspheres usually maturing. Oöspores eccentric; single; spherical; filling the oögonium; 11–24 μ in diam., predominantly 19–21 μ. Antheridial branches usually monoclinous, occasionally diclinous or androgynous. Antheridial cells laterally or apically appressed.

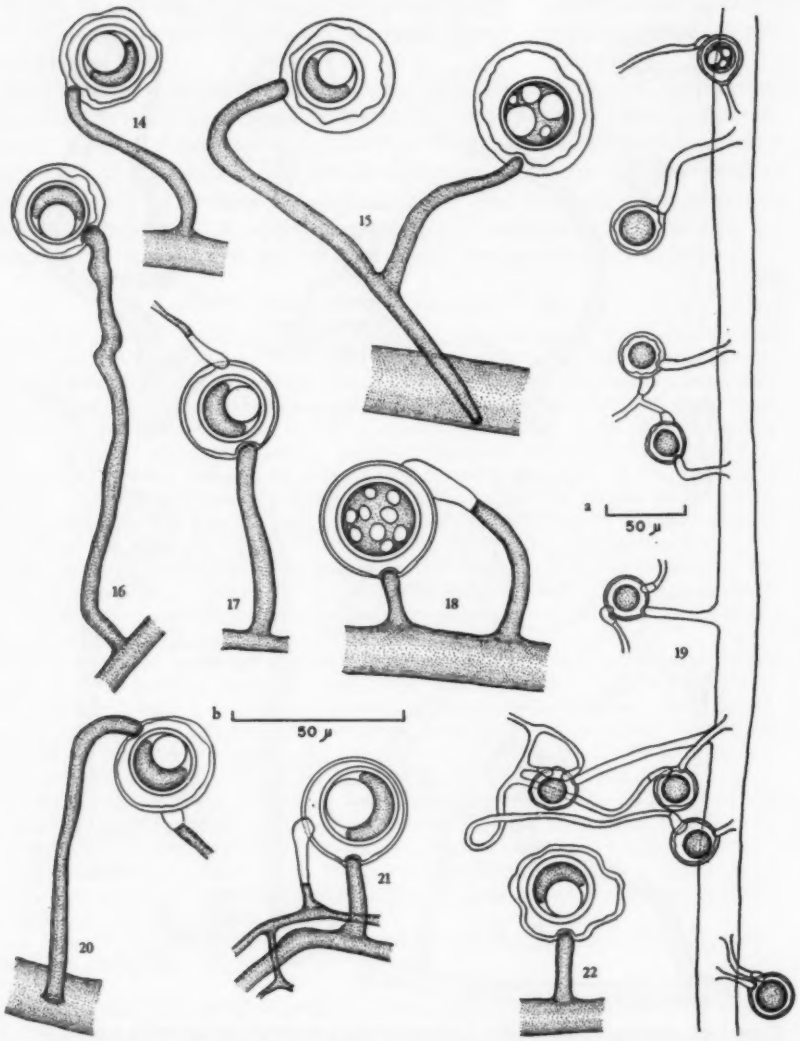
Soil, near Manati River, Manati, Barrio Rio Arriba Saliente, Dec. 31, 1954, TYPE; Vega Baja, Dec. 31, 1954.

Among the morphological characteristics which distinguish *Brevilegnia crassa* from other species in the genus two are outstanding: the extremely thick oögonial wall, and the oöspores which completely fill the oögonium (Figs. 2, 3). Empty oögonia, oögonial proliferation, and lateral hyphal swellings are other distinctive characteristics.

Considering the number of rows of zoospores, *Brevilegnia crassa* is suggestive of *B. linearis* and *B. unisperma* var. *delica* (Coker, 1927). The species is also very similar to *Brevilegnia irregularis*, but may be distinguished from the latter by reason of the thick oögonial wall. Antheridial branch origin is similar to that



FIGS. 1–13. *Brevilegnia crassa*. FIG. 1. Configuration of sexual reproductive organs. FIG. 2. Immature oögonium. FIG. 3. Oögonium with a mature oöspore. FIGS. 4, 5. Oögonia and attendant antheridial branches. FIGS. 6–9. Oögonia showing the thick wall. FIG. 10. Long-stalked oögonium and attendant monoclinous antheridial branch. FIG. 11. Zoosporangium. FIG. 12. Zoospore discharge. FIG. 13. Long-stalked oögonium with an attendant diclinous antheridial branch. FIGS. 1, 11, 12, scale b; others, scale a.



FIGS. 14–22. *Brevilegnia irregularis*. FIGS. 14–17. Oögonia showing irregular inner surface of wall. FIG. 18. Oögonium with a smooth inner surface wall. FIG. 19. General configuration of sexual reproductive organs. FIGS. 20, 21. Oögonia with attendant diclinous antheridial branches and eccentric oöspores. FIG. 22. Irregular oögonium. FIG. 19, scale a; others, scale b.

of *B. megasperma* var. *brevicaulis*, but diclinous and androgynous branches predominate in *B. crassa*.

Brevilegnia irregularis n. sp. (Figs. 14–22)

Myceliis densis; nullis gemmis. Zoosporangiis copiosis, clavatis aut fusiformibus aut naviculatis; quandoque cymosis; sympodialiter renovatis. Zoosporarum emissione brevilegnioida, quandoque dictyoida. Oogoniis copiosis, lateralibus, interdum terminalibus; sphaericis, interdum pyriformibus, subglobosis, aut irregularibus; 20–46 μ, plerumque 26–30 μ in diam. Tunicis tenuibus, hyalinis, non-punctulatis; superficie interiore levi, quandoque papillata, raro aspera; superficie interiore saepius irregulari, nonnumquam levi. Oogoniorum ramulis longis et gracilibus; rectis, quandoque curvis vel flexis, saepe cymosis; vulgo proliferentibus immaturibus. Tumoribus hyphalibus copiosis, hyalinis aut dense protoplasmicis, qui oogoniis similes videntur. Oosphaeris aut maturantibus aut non maturantibus. Oosporis excentricis, sphaericis, quae singulis in oogoniis gignuntur sed oogonia non impleant; 14–34 μ, plerumque 19–22 μ in diam. Antheridiis plerumque diclinibus, raro monoclinibus, rarissime androgenibus. Antheridiorum cellis lateraliter aut apice affixis.

Mycelium dense; principal hyphae slender. Gemmae lacking, except in old cultures. Zoosporangia abundant; clavate, fusiform, or naviculate; occasionally branched; renewed sympodially; predominantly 252–288 × 18–31 μ; generally with 2–3 rows of zoospores. Zoospore discharge brevilegnioid, occasionally dictyoid. Oögonia abundant; lateral, occasionally terminal; spherical, occasionally pyriform, subglobose, or irregular; 20–46 μ in diam., predominantly 26–30 μ; proliferation frequent. Oögonial wall thin, unpitted; outer surface smooth or occasionally sparsely papillate, infrequently verrucose; inner surface of wall usually irregular. Oögonial stalks slender; usually 3–4 times the diameter of the oögonium in length; straight, occasionally curved or bent, frequently branched. Hyaline or densely protoplasmic hyphal swellings abundant. Oöspheres generally maturing. Oöspores eccentric; single; spherical; not filling the oögonium; 14–34 μ in diam., predominantly 19–22 μ. Antheridial branches usually diclinous, infrequently monoclinous, very rarely androgynous. Antheridial cells laterally or apically appressed, occasionally digitate.

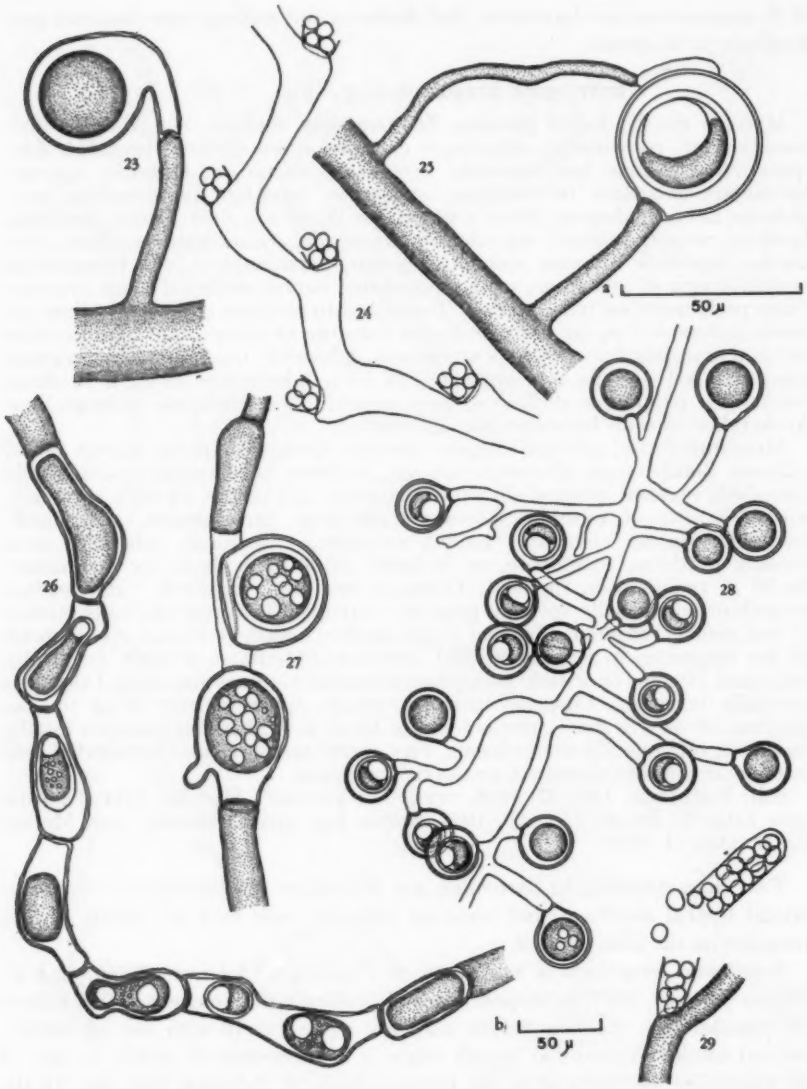
Soil, Vega Baja, Dec. 27, 1954, TYPE; "El Yunque", Dec. 29, 1954; Fajardo, near Luquillo Beach, Dec. 29, 1954; Barrio Rio Arriba Saliente, near Manati River, Dec. 31, 1954.

Two characteristics, in particular, are distinctive for *Brevilegnia irregularis*: lateral hyphal swellings, and unpitted oögonial walls that are nearly always irregular on the inner surface.

Brevilegnia irregularis is similar to *B. longicaulis* (Johnson, 1950), and *B. diclina* (Harvey, 1927) in oöspore size and proliferation of oögonia. With respect to oögonial size, the new fungus does not agree closely with any of the described species. Antheridial branch origin in *B. irregularis* is similar to that of *B. longicaulis*, particularly in the preponderance of diclinous branches. In the former, however, monoclinous branches are produced; such an origin does not occur in *B. longicaulis*. Diclinous antheridial branches are sparingly produced by *B. diclina*; *B. irregularis* consistently produces abundant antheridial branches.

Brevilegnia megasperma var. **brevicaulis** n. var. (Figs. 23–29)

Myceliis densis; gemmis copiosis. Zoosporangiis copiosis; clavatis aut fusiformibus aut naviculatis, raro filiformibus; sympodialiter, saepe cymose re-



FIGS. 23-29. *Brevilegnia megasperma* var. *brevicaulis*. FIG. 23. Pyriform oögonium. FIG. 24. Sympodial renewal of an abnormally large hypha. FIG. 25. Unpitted oögonium with an attendant monoclinous antheridial branch. FIGS. 26, 27. Intercalary, catenulate oögonia. FIG. 28. General configuration of sexual reproductive organs. FIG. 29. Zoospore discharge. FIGS. 24, 28, 29, scale b; others, scale a.

novatis. Zoosporarum emissione brevilegnioida, raro dictyoida. Oogoniis copiosis; pyriformibus, nonnumquam sphaericis, raro unam vel plures papillas habentibus, aut irregularibus; lateralibus, saepe intercalaribus et tunc singular aut catenatis, raro terminalibus; plerumque 29–32 μ in diam. Tunicis levibus, raro papillatis; non-punctulatis. Oogoniorum ramulis crassis; sessilibus. Rarissime proliferentibus immaturis. Oosphaeris maturantibus. Oosporis excentricis; sphaericis, quae singulae singulis in oogoniis gignuntur, raro binis in oogoniis intercalaribus; oogonium non implentibus; 16–32 μ, plerumque 22–24 μ in diam., 23–27 μ in oogoniis intercalaribus. Antheridiis plerumque monoclinibus, raro androgenibus aut hypogenibus, rarissime diclinibus. Antheridiorum cellis clavatis aut tubulatis; lateraliter aut apice affixis, raro digitatis.

Mycelium dense; principal hyphae slender. Gemmae abundant; filiform dolioform, or pyriform, occasionally spherical or irregular; terminal or intercalary, single or catenulate; germinating directly into new hyphae, or functioning as zoosporangia. Zoosporangia abundant; clavate, fusiform, or naviculate, infrequently filiform; renewed sympodially or cymosely; predominantly 158–200 × 22–29 μ; straight or curved, infrequently branched; usually with 2–3 rows of zoospores. Zoospore discharge brevilegnioid, infrequently dictyoid. Oögonia abundant; pyriform, occasionally spherical, infrequently with one or more papillate projections, or irregular; lateral, frequently intercalary, then single or catenulate; infrequently terminal or sessile; 21–43 μ in diam., predominantly 29–32 μ; immature ones very rarely proliferating. Oögonial stalks stout; as long as to twice the diameter of the oögonium in length; straight or curved, infrequently branched. Oögonial wall smooth, unpitted. Oöspheres maturing. Oöspores eccentric; single; spherical; rarely 2 in intercalary oögonia; not filling the oögonium; 16–32 μ in diam., predominantly 22–24 μ; these in intercalary oögonia usually 22–37 μ in diam. Antheridial branches generally monoclinous, infrequently androgynous or hypogynous, very rarely diclinous. Antheridial cells laterally or apically appressed, infrequently digitate.

Soil, roadside ditch, Road No. 3, near Canovanas, Dec. 29, 1954, TYPE; Barrio Rio Arriba Saliente, near Manati River, Dec. 31, 1954; Barrio Espinosa, Dorado, near Vega Alta, Dec. 31, 1954.

The oögonia and oöspores of *Brevilegnia megasperma* var. *megasperma* (Harvey, 1930) are generally larger than in the variety *brevicaulis*. The oögonial stalks in the new variety are generally short and stout; in the variety *megasperma*, they are predominantly long and slender. The antheridial branches of *B. megasperma* var. *brevicaulis* are mostly monoclinous, whereas Harvey reported only androgynous and hypogynal antheridia for his species. In *B. megasperma* var. *megasperma* intercalary oögonia seldom occur, whereas in the variety *brevicaulis* they are common.

OTHER FUNGI RECOVERED

Achlya americana Humphrey. Mameyes; "El Yunque"; Vega Alta; Rio Piedras; Barrio Los Hoyos. The characteristic short oögonial stalks were not observed in the isolates, and in two of these, the oöspore size is considerably smaller than reported for the species. Androgynous and diclinous antheridial branches are common.

Achlya conspicua Coker. "El Yunque." Coker (1923), Miss Crooks (1937), and Beneke (1948) did not observe mature oöspores in their isolates of this species, but on the basis of other characteristics, they concluded that the affinities

were with the group of species having subcentric oöspores. Johnson (1951), lacking evidence to the contrary, followed the disposition proposed by Coker, but stated that *A. conspicua* was most closely related to the *A. americana-klebsiana-prolifera* group. Since the isolates of *A. conspicua* from Puerto Rico produced eccentric oöspores, the species is relegated to its proper position in the subgenus *Achlya* (*Euachlya* of Coker, 1923).

Achlya dubia Coker. Barrio Monacillo; Rio Piedras. The predominant oögonial and oöspore size in the isolate is smaller than that reported by Coker (1923), and monoclinous antheridial branches are present. The oögonial walls, according to Coker, are pitted only under the point of attachment of the antheridial cells, but in the isolate examined, the walls are pitted at other points as well. The number of oöspores in the Puerto Rico isolate ranges from 1–15; Coker reported the range as 2–8. The fungus consistently exhibits thraustothecoid primary zoosporangia, the principal diagnostic characteristic for the species.

Allomyces javanicus Kniep. Barrio Monacillo; Rio Piedras; Guaynabo; Barrio Rio Arriba Saliente; Barrio Espinosa. Parthenogenetic development of the female gametes into new sporothalli was reported by Kniep. Four of the Puerto Rico isolates exhibited this phenomenon, and male gametangia were entirely lacking.

Rozella allomycis Foust. Parasitic in *Allomyces arbusculus*, isolated from soil, Barrio Los Hoyos. The resting spores are black, and not yellow- or red-brown as reported by Miss Foust (1937). Resting spore wall ornamentations are stout and conical.

Some species were collected, the isolates of which did not vary significantly from previous descriptions. These taxa, not requiring a discussion, include the following. *Achlya diffusa* Harvey: "El Yunque." *Achlya flagellata* Coker; "El Yunque." *Achlya klebsiana* Pieters; Barrio Espinosa, Barrio Rio Arriba Saliente. *Achlya prolifera* Nees; Bayamón River, Bayamón. *Dictyuchus anomalus* Nagai; Barrio Espinosa. *Saprolegnia delica* Coker; "El Yunque." *Allomyces arbusculus* E. J. Butler emend. Hatch; Barrio Los Hoyos, Bayamón River, Bayamón. *Allomyces cystogenus* var. *cystogenus* Emerson; Vega Baja, Rio Piedras, Barrio Rio Arriba Saliente.

SUMMARY

Fifteen species, representing five genera of filamentous aquatic Phycomycetes were isolated from Puerto Rico soil. *Brevilegnia crassa* and *B. irregularis* are proposed as new species; *B. megasperma* var. *brevicaulis* is described as a new variety.

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LITERATURE CITED

BENEKE, E. S. 1948. A monographic study of Illinois species of the Saprolegniales and Leptomitales. (Unpublished doctoral dissertation, Univ. Illinois.)

COKER, W. C. 1923. The Saprolegniaceae. Univ. North Carolina Press: Chapel Hill.

———. 1927. Other water molds from the soil. Jour. Elisha Mitchell Sci. Soc. **42**: 207–266.

———, AND V. D. MATTHEWS. 1937. Saprolegniales. North Amer. Flora **2**(1): 15–67.

CROOKS, KATHLEEN M. 1937. Studies on Australian aquatic Phycomycetes. Proc. Royal Soc. Victoria (N.S.) **49**: 206–232.

FOUST, FRANCES K. 1937. A new species of *Rozella* parasitic on *Allomyces*. Jour. Elisha Mitchell Sci. Soc. **53**: 197–204.

HARVEY, J. V. 1927. *Brevilegnia diclina* n. sp. Jour. Elisha Mitchell Sci. Soc. **42**: 243–246.

———. 1930. A taxonomic and morphological study of some members of the Saprolegniaceae. *Ibid.* **45**: 319–332.

JOHNSON, T. W., JR. 1950. A study of an isolate of *Brevilegnia* from New Caledonia. Mycologia **42**: 242–252.

———. 1951. Taxonomic studies in the genus *Achlya* Nees. (Unpublished doctoral dissertation, Univ. Michigan.)

SPARROW, F. K., JR. 1943. Aquatic Phycomycetes. Univ. Michigan Press: Ann Arbor.

NESTRONIA, PARASITIC ON ROOTS OF PINUS

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The chiefly tropical family, Santalaceae, is represented in the southeastern United States by four genera of one species each: namely, *Commandra umbellata* (L.) Nutt., *Pyrularia pubera* Michx., *Nestronia umbellata* Raf. and *Buckleya distichophylla* Torr. All are parasitic on the roots of other plants and only one, *Buckleya*, is considered as parasitic on the roots of coniferous trees, and this only on *Tsuga*. The genus *Nestronia* is represented in the world by only one species. Both Fernald and Small give as its range uplands of Virginia to Georgia and Alabama. Fernald, in *Gray's Manual of Botany*, eighth edition, 1950, states: "Parasitic on roots of deciduous trees." Small, in his *Manual of the Southeastern Flora*, says: "This shrub, unlike *Buckleya*, is, as far as we know, parasitic on the roots of the deciduous-leaved shrubs and trees with which it grows. Coniferous trees are often wanting there."

A very limited stand of *Nestronia* was found on the south bank of Deep River above Freeman's Mill in Guildford County, North Carolina, on September 2, 1955, growing under *Pinus echinata* and hardwoods. On my return to the location two days later I took up three portions of the clump and brought them home, where all soil around the roots was removed with the aid of a water hose. In two of these partial clumps no attachment was found of the roots of *Nestronia* to those of other plants, but in the third definite attachment was found to the

root of pine. This specimen was preserved and presented to the herbarium of the University of North Carolina (NCU *74195*).

I believe this to be the first evidence offered that *Nestronia* may be parasitic on the roots of a coniferous tree. The presence of many unattached roots found on the *Nestronia* indicates that the plant is not totally parasitic and may obtain part of its nourishment independently of other plants.

THE EFFECT OF MALEIC HYDRAZIDE ON THE GROWTH OF TWENTY-TWO SPECIES OF FUNGI[1]

BY VICTOR A. GREULACH AND CHARLES E. MILLER

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Subsequently to the discovery by Schoene and Hoffmann (1949) of the growth-inhibiting properties of maleic hydrazide (MH) or 3,6-pyridazinedione, 1,2-dihydro on tomatoes, numerous investigators have conducted studies which indicate that MH is probably a universal growth inhibitor for vascular plants, if not for all green plants. On the other hand, Greulach *et al.* (1951) found that MH did not inhibit the growth of Amphibian embryos, mice, or testicular tumors of mice, while Tate (1955) reported that MH did not inhibit the growth of rats or dogs. Since MH is an anti-mitotic substance it must act on metabolic processes involved in plant, but not animal, mitosis.

So far there is little information on the effect of MH on the growth of the third major type of organisms—the non-green plant. Klein and Klein (1952) reported that MH inhibited, but did not prevent, the multiplication of *Agrobacterium tumefaciens*, while Waggoner and Dimond (1955) found that 28.8×10^{-3} MH did not inhibit this species. Nickell and English (1953) studied the effects of MH on *Candida albicans* and on several different species of bacteria, in particular various species of soil bacteria, and found that 100 p.p.m. MH had no effect on any of the species. *Rhizobium* was inhibited by 280 p.p.m., while other genera were inhibited only by 1000 p.p.m. or more. O'Brien and Bornmann (1950) found that 0.05% MH prevented the growth of the fungus *Alternaria solani* beyond a short germ tube. The interest of the writers in the possible effects of MH on fungi was originally stimulated by finding flourishing colonies of an unidentified Ascomycete in a 4% stock solution of the diethanolamine salt of maleic hydrazide. This high concentration would not only inhibit the growth of higher plants, but would also be lethal, while the fungus was apparently utilizing the MH as both a carbon and a nitrogen source, and thriving on this unusual food.

METHODS.—In this study 22 different species of fungi were tested for their growth responses to MH. The fungi were cultured in 9-cm. Petri dishes on a

[1] The authors wish to thank Dr. J. N. Couch and Dr. Kathleen Goldie-Smith for providing inoculum of ten of these species from their stock cultures.

yeast extract medium (yeast extract, 2.5 g.; glucose, 15.0 g.; agar, 15 g.; and water, 1 liter). The two test series were prepared by adding 2000 or 5000 p.p.m. of pure maleic hydrazide to the above medium. Both the control and test media were buffered by means of 0.6 g. K_2HPO_4 plus 0.4 g. KH_2PO_4 per liter, the pH in all three media being 6.8 after autoclaving. A preliminary test of autoclaved and un-autoclaved MH on bean plants showed that autoclaving did not destroy its growth-inhibiting properties or even reduce them. The osmotic pressure of a 2000 p.p.m. solution of MH was found to be 0.64 atmos. and that of the 5000

TABLE I

Effect of maleic hydrazide on the growth of several fungi

Group[1]	Species	Class[2]	Growth index[3]	Growth of treated plants as per cent of growth of controls	
				2000 p.p.m. MH	5000 p.p.m. MH
I	*Pythium* sp.	P	8	9.0	7.9
	Apodachlya sp.	P	17	26.1	7.7
	Saprolegnia diclina	P	20	21.3	18.3
	Thraustotheca clavata	P	28	38.3	17.4
II	*Hyphochytrium catenoides*	P	36	52.0	20.0
	Achlya hypogyna	P	45	55.2	35.5
	Aphanomyces laevis	P	59	68.3	50.6
	Psalliota campestris	B	67	70.0	63.3
	Allomyces javanicus	P	74	78.5	70.2
	Coprinus sterquilinus	B	80	80.4	79.3
III	*Mycotypha dichotoma*	P	84	94.2	73.5
	Monascus purpureus	A	86	97.2	75.3
	Endomyces magnusii	A	88	94.2	81.4
IV	*Geolegnia inflata*	P	96	95.7	95.7
	Dictyuchus sp.	P	97	96.5	96.5
	Ascocybe grovesii	A	99	100.0	97.8
	Rhizopus nigricans	P	105	102.8	107.1
	Ascosphaera apis, ♂	A	106	105.9	105.9
V	*Penicillium notatum*	A	109	106.6	112.1
	Mucor racemosus, −	P	111	107.1	114.2
	Mucor racemosus, +	P	115	108.9	120.8
	Ascosphaera apis, ♀	A	116	113.3	118.6
	Pyronema sp.	A	118	114.0	121.0
	Sordaria fimicola	A	152	140.7	162.9

[1] I—Marked growth inhibition; II—Inhibition by both 2000 and 5000 p.p.m. MH; III—Inhibition by 5000 p.p.m. MH only; IV—No inhibition; V—Promotion of growth by MH.

[2] A—Ascomycete; B—Basidiomycete; P—Phycomycete

[3] Mean of per cent of growth of controls of plants treated with 2000 and 5000 p.p.m. MH, rounded off to nearest whole per cent

p.p.m. MH 1.60 atmos. Although it seemed obvious that this was not sufficient to have an adverse osmotic effect on the fungi, the possibility was definitely eliminated by finding that lactose solutions of these osmotic pressures did not affect the growth of the fungi.

The Petri dishes were inoculated by placing a block of agar containing the hyphae in the center of the dishes, each agar block being 2 mm. square. The cultures were maintained in a room kept at 20°C. by air conditioning, under ordinary indoor light conditions.

The amount of growth of each colony was determined by measuring its diameter. Daily measurements were made, permitting the construction of growth curves, which can not be reproduced here because of space limitations. Some species were measured over a period of a month, but with most species the most rapidly growing colony reached the edge of the dish much sooner than this, the time being only 2 days for *Rhizopus nigricans* and 4 days for *Pythium sp.* Because of the great difference in growth rate of the different species, growth of the treated plants was expressed as per cent of growth of the controls, providing comparable data for the different species. The data in Table I are based on the sizes of the treated colonies as compared with the controls at the time when the most rapidly growing of the three colonies had reached a point 1 cm. from the edge of the dish. Where none of the colonies attained this size the final measurements of colony size were used.

RESULTS.—The results of the tests are summarized in Table I, the species being arranged in the order of the amount of growth inhibition caused by the MH, on the basis of a somewhat arbitrary growth index. This growth index is the mean, rounded off to the nearest whole number, of the per cent of growth inhibition by 2000 and 5000 p.p.m. MH. In species whose growth was affected by MH the effect appeared promptly—within one or two days after inoculation in most species and never more than four days after inoculation. The limits of the five groups of fungi as regards their reaction to MH were determined more or less arbitrarily, since the data secured were not susceptible to statistical analysis.

DISCUSSION.—It is obvious that the fungi tested vary greatly in their response to MH, the marked extremes being inhibition by 5000 p.p.m. MH in *Pythium sp.* to only 7.9 per cent of the growth of the controls and the promotion of growth by 5000 p.p.m. MH in *Sordaria fimicola* to 162.9 per cent of the controls. That the MH actually promoted growth of the species in group V is substantiated by the fact that in each species in this group growth was more rapid with 5000 p.p.m. MH than with 2000 p.p.m. MH, just the reverse of the situation in the species in which MH inhibited growth. This great variation in the response of the different species of fungi to MH is in line with the general diversity of metabolism in different species of bacteria and fungi, in contrast to the general metabolic uniformity within the green plants and within the animal kingdom.

In general, it appears that Phycomycetes are more likely to have their growth inhibited by MH, while the growth of the Ascomycetes is in general either not affected or promoted by MH. However, there are exceptions to this generalization even among the relatively few species tested and if more species were

investigated the distinction might disappear. Not enough Basidiomycetes were tested to permit even this degree of generalization about this class. It does appear worth noting that the species in group V, whose growth was promoted by MH, are in general vigorously growing molds which thrive under a great variety of environmental conditions, many of them quite adverse. This is probably the first report of a growth-stimulating effect of MH, but it appears possible that the MH is serving as a nutrient rather than as a growth substance. Since the yeast extract was the only source of nitrogen in the culture medium, it appears possible that the group V plants were able to metabolize the MH and use it as a nitrogen source.

If this supposition is correct, there would be only two basic classes as regards response to MH for the fungi tested: (a) Those in which growth is inhibited to a greater or lesser degree by MH (groups I, II and III), and (b) those which are not inhibited by MH (groups IV and V). The second class would include fungi which cannot metabolize and use MH (group IV) and those which can (group V).

A survey of the literature has failed to reveal any general or consistent metabolic differences between fungi of the various groups which would correlate with their responses to MH. Additional more intensive studies on the effects of MH on the metabolism of fungi selected from each of the five groups, or at least from groups I, IV and V, should help in further clarifying the mode of MH action in inhibiting growth and would probably also provide fundamental information about the growth and metabolism of the species studied. If, as Leopold and Klein (1952) and several other investigators propose, MH acts as an anti-auxin, the fungi in the first three groups would be expected to require auxin for growth while the last two groups would not.

SUMMARY

Twenty-two species of fungi were cultured in Petri dishes on agar incorporating a yeast extract medium buffered to pH 6.8 by means of phosphate buffers, and with 0, 2000 and 5000 p.p.m. maleic hydrazide added to the culture medium. The various species differed greatly in their reactions to MH, ranging from *Pythium sp.*, which with 5000 p.p.m. MH grew only 7.9 per cent as much as the controls, to *Sordaria fimicola*, which had 162.9 per cent of the growth of the controls when 5000 p.p.m. MH was used. The species studied can be classed in two main groups: those which are inhibited to a greater or lesser degree by MH and those which are not. Some of the species in the second group are apparently able to utilize MH, probably as a nitrogen source, while others are not.

LITERATURE CITED

GREULACH, V. A., JOHN MCKENZIE AND EMILY M. STACY. 1951. The effect of maleic hydrazide on the embryonic and larval growth of three amphibians. Biol. Bull. **101**: 285–288.

KLEIN, R. M. AND DEANA T. KLEIN. 1952. Effects of maleic hydrazide on initiation and development of tomato crown-gall tumors. Amer. Jour. Bot. **39**: 727–730.

LEOPOLD, A. C. AND W. H. KLEIN. 1952. Maleic hydrazide as an anti-auxin. Physiologia Plantarum **5**: 91–99.

NICKELL, L. G. AND A. R. ENGLISH. 1953. Effect of maleic hydrazide on soil bacteria and other microorganisms. Weeds **3**: 190–195.

O'BRIEN, G. AND ADELAIDE BORNMANN. 1950. Fungicidal properties of maleic hydrazide. Unpublished report. (See MHIS 6, p. 19, Naugatuck Chemical Co.)

SCHOENE, D. L. AND O. L. HOFFMANN. 1949. Maleic hydrazide, a unique growth regulant. Science **109**: 588–590.

TATE, H. D. 1955. Summary of mammalian toxicity studies with maleic hydrazide. Unpublished report. (See MHIS 6C, p. 26, Naugatuck Chemical Co.)

WAGGONER, P. E., AND A. E. DIMOND. 1955. Assaying effect of growth regulators upon plant tumors. Conn. Agr. Expt. Sta. Bull. 587.

NATURAL HYBRIDS IN THE GENUS SARRACENIA. II. CURRENT NOTES ON DISTRIBUTION

BY C. RITCHIE BELL[1] AND FREDERICK W. CASE[2]

In the course of obtaining natural *Sarracenia* hybrids for use in the cytological investigations which were planned as the second and final phase of this project, three previously unreported natural hybrids have been found and many new localities, field data, and some experimental data have been added to the file of information available for the previous article on these plants (Bell, 1952).

The information in the following paragraphs has been accumulated over a period of only two years, but the data represent the results of the four most recent trips through the southeast by the two authors, in addition to the information and specimens received from several valued correspondents. A trip to the Gulf Coast and South Atlantic states was made by the senior author in April of 1954 and again in 1955.[3] The same general areas were visited by the junior author in June of 1954 and 1955. This arrangement, dictated by teaching schedules and entirely unplanned, has proven very satisfactory, since some *Sarracenia* hybrids are identified by their flowers and others by their leaves, which usually come along later in the season.

Because of the blooming habits of *Sarracenia* it may be several years before enough cytological information concerning hybrids in this genus is accumulated to warrant publication. In the meantime, however, especially in view of the increasing taxonomic activity in the southeast, the following notes may be of some value. They might also result, indirectly, in additional reports of localities where *Sarracenia* hybrids might be found. Reprints or further letters concerning such localities will be appreciated!

In order to facilitate cross referencing with the first paper of this series (Bell, 1952), the taxa treated below will be numbered in accordance with their number

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[3] These trips were largely financed by two generous travel grants from the University Research Board of the University of Illinois. The assistance of Mr. Harry Ahles and Mr. Robert Gilpin on these trips is gratefully acknowledged and the two newly discovered hybrids are named in their honor.

and treatment in this previous work. The two taxonomic changes, *Sarracenia alata* (Wood) Wood = *S. Sledgei* Macfarlane (Merrill, 1948) and *S. leucophylla* Raf. = *S. Drummondii* Croom (Bell, 1954) should be noted.

The first set of herbarium specimens and living plants of all hybrids collected by the senior author are at the University of North Carolina. All collections of the junior author are of living material unless it is indicated, by the appropriate abbreviation, that part or all of the collection is on file in some herbarium.

1. *S. flava* × *purpurea*
Sarracenia ×*Catesbaei* (Elliott) Bell

Though considered to be one of the more common *Sarracenia* hybrids, none of these plants was found in the Gulf Coast area, and only a few were seen in the savannahs southwest of Wilmington, North Carolina in 1954.

2. *S. leucophylla* × *purpurea*
Sarracenia ×*Mitchelliana* (Nichols.) Bell

While there are no new state records for this hybrid, plants from Florida collections have been pressed (*Bell 1501*, *1504*, Escambia Co.; *1520*, Liberty Co.) to document its occurrence in that state. Actually, this hybrid is not uncommon in many of the bogs near Pensacola and other west Florida cities (*Case P-69*) and it has also been found, often in bloom, in additional areas around Mobile, Baldwin county, Alabama (*Bell 1500*).

3. *S. minor* × *purpurea*
Sarracenia ×*Swaniana* (Wm. Robinson) Bell

One of these hybrids was collected recently near Bladenboro, North Carolina, by Dr. Carroll Wood. The plant, now growing in the Botany Greenhouse at the University of North Carolina, represents a westward range extension of about 50 miles. In addition to this hybrid another plant, which appears to be (*S. minor* × *purpurea*) × *minor*, was also collected by Dr. Wood at the Bladenboro locality.

The only collection of this hybrid was made by one of the authors with the guidance of Mr. Henry Rehder, near the type locality in Brunswick county, North Carolina. Five plants were collected, but have not bloomed. Apparently this will continue to be one of the rarer *Sarracenia* hybrids, although it has a potential range over portions of South Carolina, Georgia and Florida.

4. *S. psittacina* × *purpurea*
Sarracenia ×*Courtii* Masters

One of two, or possibly three (counting *Sarracenia leucophylla* × *minor*, which perhaps should no longer be continued in the list of possible natural hybrids; see under no. 8) natural *Sarracenia* hybrids still unreported.

There is some slight possibility, considering the strong effect of *Sarracenia psittacina* in any cross, the dimorphic leaves of these hybrids, and the rather pronounced morphological similarity of several of the different *S. psittacina*

hybrids, that this particular cross has been mistaken, in the field, for *S. psittacina* × *rubra*. If leaf form of these two hybrids is similar, general flower shape and scent will have to be used in their separation.

5. *S. purpurea* × *rubra*
Sarracenia ×*Chelsoni* Masters

Previously reported only from Scotland and Henderson counties in North Carolina, plants which appeared to be of this parentage were collected at two stations in George county, Mississippi. Plants from both stations were tentatively identified on leaf form even though there was some difference between actual leaf morphology and the description of *Sarracenia* ×*Chelsoni* leaves in the literature. These plants from near Agricola, Mississippi, bloomed in 1955 and the flowers confirmed the tentative identification of this collection (*Bell 1456 b*).

Living plants of *Sarracenia* ×*Chelsoni*, in this case *S. purpurea* × *rubra f. Jonesii* (*Case P-51*) were recollected in Henderson county, North Carolina, 1.3 miles south of Flat Rock Post Office, and others were observed near Etowah. Some of these plants are growing in a greenhouse in Saginaw, Michigan. Others are growing in a natural habitat on the estate of Mr. C. F. Moore at Brevard, North Carolina.

A third recent collection of this hybrid (*Case P-65F*) from Santa Rosa county, Florida, adds another "new state" to the known distribution of *Sarracenia* ×*Chelsoni*.

6. *S. alata* × *purpurea*
Sarracenia ×*exornata* (S. G.) Bell

Plants of this parentage were previously reported only from Mobile county, Alabama, but in a bog five miles south of Agricola, Mississippi, 228 of these hybrids were counted in an area comprising only about one-fourth of the total bog. *Sarracenia alata* is common, with *S. psittacina*, in the wet, quaking, central portion of the bog and *S. purpurea* is abundant on the low slopes around the bog and at the bog's edge. The hybrids were found, almost without exception, in a band 10 to 20 feet wide at the bog margin. From the few specimens of *S.* ×*exornata* previously examined, it seemed that these plants, in general, were smaller than plants of *S.* ×*Catesbaei*. This was considered to be caused by the difference in average size between *S. alata* and *S. flava*, the parents, other than *S. purpurea*, in the two respective hybrids. This average size difference, however, did not hold for the many *S.* ×*exornata* plants observed at the locality mentioned above. Here the plants presented a range of sizes (*Bell 1451*) possibly controlled more by environment than by genetics, quite similar to the range of sizes found in a comparison of numerous *S.* ×*Catesbaei* plants. Also noted in this series of *S. alata* × *purpurea* hybrids were several variations in hood size, margin, and coloration, possibly indicating F_2 plants or backcrosses.

Additional collections of *Sarracenia alata* × *purpurea* have been made south of Theodore, Alabama (*Case P-48, P-49; Bell 1531, 1538*), and these, too, show variation in size and form.

Another collection (*Case P-66b, P-66c, P-66d*) of *Sarracenia alata* × *purpurea* was made along Alabama Highway 3, 5.5 miles south of Foley, in Baldwin county. This collection is from the easternmost edge of the possible range of *S.* ×*exornata*, and came from a disturbed habitat where the only pure species appeared to be *S. psittacina*. Plants of the other species present showed varying effects of hybridization and presumably introgression.

7. *S. flava* × *leucophylla*
Sarracenia ×*Mooreana* (Veitch) Bell

No range extensions have been obtained for *Sarracenia* ×*Mooreana*. Additional field data and collections were obtained in Santa Rosa county (*Bell 1466, Case P-43*) and Okaloosa county (*Case P-44, P-46*), Florida.

8. *S. leucophylla* × *minor*
Sarracenia ×*excellens* (Nichols.) Bell

A search for *Sarracenia leucophylla* at the western edge of the range of *S. minor* was unsuccessful. Unless the range of *S. minor* moves westward or that of *S. leucophylla* becomes more easterly it may be that this taxon will have to be removed from the list of "geographically possible" natural hybrids.

9. *S. leucophylla* × *psittacina*
Sarracenia ×*Wrigleyana* (S. G.) Bell

Plants intermediate between parents in leaf shape and coloration, but most specimens examined tend to be more nearly like *Sarracenia psittacina* in size. Summer leaves in basal rosette are semi-erect, arching, expanded upward, wing to 1 cm. wide with widest portion near center, leaves 0.6–2.5 dm. tall (usually 0.6–1.6 dm.); hood of leaf arching over the oblique orifice, slightly globose, truncate, margins slightly, if at all, undulate, red veins often prominent against white of the hood and upper portion of pitcher, or, in shaded plants, veins subdued and hood coloration more green than white. Scape 3.5 dm. tall. Flowers odorless; sepals ovate, 3.5 cm. long by 2.8 cm. wide, maroon; petals obovate, 6 cm. long by 2 cm. wide at lobe, maroon.

TYPE LOCALITY: Fowl River Road south of Theodore, Mobile county, Alabama. 1954.

POSSIBLE RANGE: In association with parent species in limited areas of southwestern Georgia and more widely in northwestern Florida and southern Alabama west into southeastern Mississippi.

SPECIMENS EXAMINED: Alabama. Fowl River, Mobile Co., *Case P-62* (NCU: type); Fruitdale, Washington Co., *Wherry, July 1932* (PENN). Florida. Pensacola, Escambia Co., *Bell 1505* (NCU).

This striking hybrid, previously described only from horticultural material and known as a natural hybrid from only one reported locality in Florida (Bell, 1952 p. 69), was found south of Theodore near Fowl River in Mobile county, Alabama, by the junior author in 1954 (*Case P-62*). It has since been collected also in Escambia county, Florida (*Bell 1505*), and a previous collection of this

plant by Dr. E. T. Wherry has come to light. This collection, from "4 miles southeast of Fruitdale," Washington county, Alabama, was made on July 12, 1932. In neither of the first two cases were the hybrids present just as single plants: at the Fowl River locality there were estimated to be 50 of these plants, and there were 10 of them seen in the bog along Florida Highway 297 just west of Pensacola.

The plants of this hybrid (Fig. 4b) observed in the field were, with one exception, much smaller than would be expected from the horticultural descriptions which gave a leaf size of "twelve inches to fifteen inches." The one larger plant, collected at the Fowl River locality, had some leaves up to the 12-inch mark but none larger. Measurements from this plant account for the larger measurements given in the above description, and, unless all of the other sixty or more plants observed and collected are to be considered as all being in the same state of immaturity, these larger measurements are to be considered the exception rather than the rule.

10. *S. leucophylla* × *rubra*
Sarracenia ×*Readii* Bell

Scape 3.3–5.9 dm. tall, equalling the length of the leaves. Flowers intermediate in size, with a very faint sweet scent; sepals ovate, maroon; petals obovate, maroon; style disc green.

Previously known only from the type collection, this interesting hybrid was recently recollected in Washington county, Alabama, in the general area of the type locality (*Bell 1496; Case P-63*). Collections of this hybrid were also made in Santa Rosa county, Florida (*Bell 1526; Case P-42*), and thus document an earlier report of *Sarracenia* ×*Readii* by Macfarlane (Bell 1952, p. 69). Two of the plants of collection *1496* were in bloom and these, with two blooms on *P-42* (under cultivation), furnished the first information on floral characteristics that we have for this hybrid. The maroon flowers are intermediate in size between the robust flowers of *S. leucophylla* and the small, delicate flowers of *S. rubra* (or, under cultivation, may equal the size found in the larger parent). Some of the green coloration on the inner petal surface and a very faint sweet scent can be attributed to the effect of the *S. rubra* genes in the hybrid.

Under cultivation the new leaves of these hybrids are longer, often less mottled with white around the hood and more like *Sarracenia rubra* leaves; much of the *S. leucophylla* aspect, other than size and the undulate hood has been lost.

11. *S. leucophylla* × *alata*
Sarracenia ×*areolata* (Macfar.) Bell

This hybrid is common in the Mobile area and known to all who have collected there. It is the only natural hybrid in the genus which occurs in such numbers, over a relatively large area and at enough different locations, to make practical a statistical analysis of the many forms to determine, if possible, the extent and probable direction of introgression. A start on one aspect of such an analysis is being made by the junior author in an attempt to get some information on

Sarracenia rubra, the only species (as presently defined) in the genus which approaches a "species complex."

Among the many backcrosses and F_2 plants from original *Sarracenia alata* × *leucophylla* crosses are some plants which have the leaf characteristics of *S. alata*, but which have light to dark maroon flowers. The developing leaves of these plants nearly always show faint areoles, later obscured, which do not exist in any form of *S. rubra*. Such plants, especially near Mobile, have probably led to erroneous reports of *S. rubra* and *S. rubra* f. *Jonesii* in this area. The junior author has made five trips to the Mobile area and has been unable to find there any plants referable to the *S. rubra* complex.

12. *S. flava* × *minor*
Sarracenia ×*Harperi* Bell

Scape 2.3–3.5 dm. tall, equallying length of leaves. Flowers of medium size, 6–11 cm. across when pressed, with a very faint but definite musty scent; sepals ovate, yellow-green; petals 4.5–5.5 cm. long, drooping, clear yellow; style disc 3–4 cm. in diameter, green.

Several clumps of this hybrid have been found at new localities; but of greater interest than the moderate range extension in Georgia (*Bell 1507*, Thomas Co.) and the documentation (*Bell 1486*, Charleston Co.) of the reported occurrence of this plant in South Carolina (Harper 1918) is the fact that both of these collections were of flowering material. There are no previous records of flowers on any plants of this hybrid, and Harper (1918) had considered it sterile.

Early in 1955 Dr. E. T. Wherry sent for examination a plant of *Sarracenia flava* × *minor*, just out of storage, which he had collected on July 20, 1932, in Hampton county, South Carolina, and was in fruit. This specimen further documents the occurrence of *S.* ×*Harperi* in South Carolina, as well as its potentially fertile nature.

13. *S. minor* × *psittacina*
Sarracenia ×*formosa* Veitch ex Masters

Scape 2.0–3.5 dm. long, slightly taller than leaves. Flowers odorless; sepals ovate, 2–3 cm. long by 1.1–1.7 cm. broad, green, tinged with maroon; petals obovate, 3.5–4 cm. long by 1.5–2.0 cm. broad at lobe, light maroon and yellow outside, yellow-pink inside.

Although this hybrid has been collected in fruit, its floral characters have until now been unknown. The first collection of this hybrid in nearly fifty years (*Bell 1482*) was a bountiful one, as over twenty of these plants, in bud and bloom, were found 5 miles west of Callahan in Nassau county, Florida. More recently two of these hybrids were found, with many plants of *Sarracenia minor* but only one clump of *S. psittacina*, near Hoobs Church in Thomas county, Georgia. Both of these collections represent range extensions; the former collection (*Bell 1482*) is a new state record for Florida.

Plants of this hybrid grown for one year in the greenhouse have given the answer to the puzzling differences in leaf morphology shown by comparing a

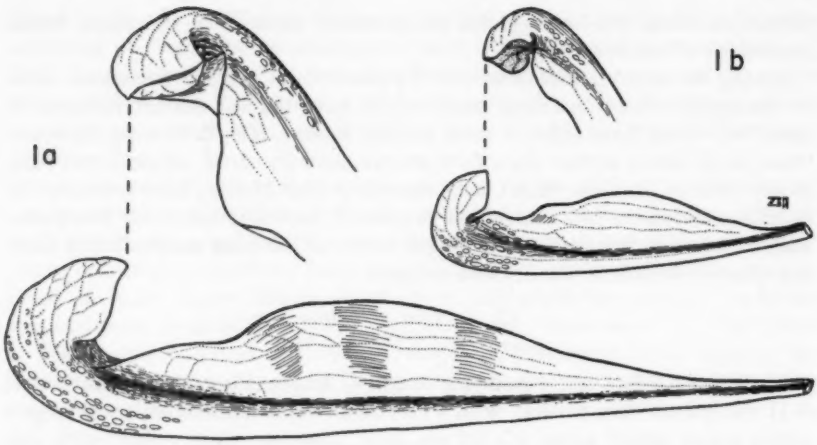

FIG. 1. Variation in leaf size and hood form in *Sarracenia minor* × *psittacina* under (a) greenhouse conditions, and (b) field conditions.

picture of this hybrid as a horticultural plant with an herbarium specimen of this plant as collected in the field (Bell, 1952; Plate 6, 1–2.) This dimorphic leaf condition, noted to a greater or lesser degree in all hybrids involving *Sarracenia psittacina*, is most pronounced in *S.* ×*formosa* and so will be discussed briefly at this point.

Some factor of greenhouse culture, most likely heat or light, or both, influences these plants in such a way that the leaves are not only longer than usual, but they have an enclosed hood with a small psittacina-like opening into the pitchered leaf, as shown in the two views of one of these leaves in figure 1a. Under field conditions the weak adaxial suture of the hood splits and allows the hood to flare, in a manner somewhat like that of the hood of *Sarracenia minor*, and thus form a larger orifice, as shown in figure 1b.

In three dimensions the truncate, bonnet-shaped hoods found on field-grown plants are entirely different, in over-all appearance, from the inflated, enclosed, pointed or beak-like hoods of the plants grown in the greenhouse. Because both kinds of leaves are on a single plant after one season in the greenhouse, and sometimes in the field, this difference cannot be considered a response to differences in genetic makeup.

14. *S. minor* × *rubra*
Sarracenia ×*Rehderi* Bell

Only one additional collection of this plant has been made. It was made, with the guidance of Mr. Henry Rehder, from the general area of the type locality. So far no additional localities are known.

15. *S. flava* × *rubra*
Sarracenia ×*Popei* Masters

No additional plants of this hybrid have been found; the neotype *Harriot 815520* (PH) is still the only known collection of this plant as a natural hybrid, despite the rather large areas of the southeast in which it can reasonably be expected to occur.

16. *S. psittacina* × *rubra*

Sarracenia ×Gilpini hybr. nov.

Sarracenia ×Gilpini, hybrida naturalis nova inter S. psittacinam et S. rubram, foliis sub-erectis arcuatis rubro-reticulatis 0.4–1.6 dm. altis, cucullo globato acuminato aut truncato, minutis floribus odoriferis et purpureis.

Plants intermediate between parents. Leaves semi-erect, from basal rosette; 0.4–1.6 dm. in length, tapered from base to orifice, arching distally, yellow-green or green, often heavily shaded or reticulated with maroon and with more or less prominent white areoles on abaxial surface and around orifice, adaxial wing linear or rarely expanded, widest near middle; hood inflated, pointed, folded over slit-like orifice, or, if the weak adaxial suture ruptures the hood may be expanded, truncate, bonnet-shaped. (See Fig. 2, 4d.) Scape exceeding the height of the leaves. Flowers small, faintly sweet-scented; sepals ovate, 1.5–2.0 cm. long by 1.0–1.3 cm. broad, maroon outside, green edged with maroon inside; petals obovate, 3 cm. long by 1.6 cm. broad at broadest part, maroon; style disc 2.0 cm. in diameter, green.

TYPE LOCALITY: Savannah north of Yellow River and east of Florida Highway 87; Santa Rosa Co., Fla. 1955.

POSSIBLE RANGE: In association with parent species in southern Georgia and northern Florida west through southern Alabama into southeastern coastal Mississippi.

SPECIMENS EXAMINED: Florida. Yellow River, Santa Rosa Co. *Bell 1523* (NCU: type), *Case P-65a.*

This small hybrid, previously unreported either as a natural or as an artificial hybrid, was first collected in April, 1955, by the senior author. Forty-eight of these plants were counted in the area of the type locality and ten were collected. Some of the plants were in bud or bloom and all of the latter had small flowers, with a faint sweet scent, and strongly obovate petals—these three characteristics mark *Sarracenia rubra* as one of the parents rather than *S. leucophylla* or *S. purpurea*, two other red-flowered species which were also present in the immediate area. The arch of the leaves, the coloration and form of the hood, and the long hairs inside and near the base of the hollow leaf indicate only *S. psittacina*. The strigose adaxial surface of the hood is an anomalous feature of these hybrids, as represented by the type collection, because by extrapolation the hybrid would be expected to have a hood with an adaxial surface intermediate in hirsuteness between the puberulent condition of typical *S. rubra* and the thinly sericeous (usage according to Lawrence, 1951 pp. 746–7) condition found in normal *S. psittacina*. The anomaly may be explained by the fact that the leaf hoods of all of the *S. rubra* (*Bell 1522*) collected at the type locality of *S.* ×*Gilpini*

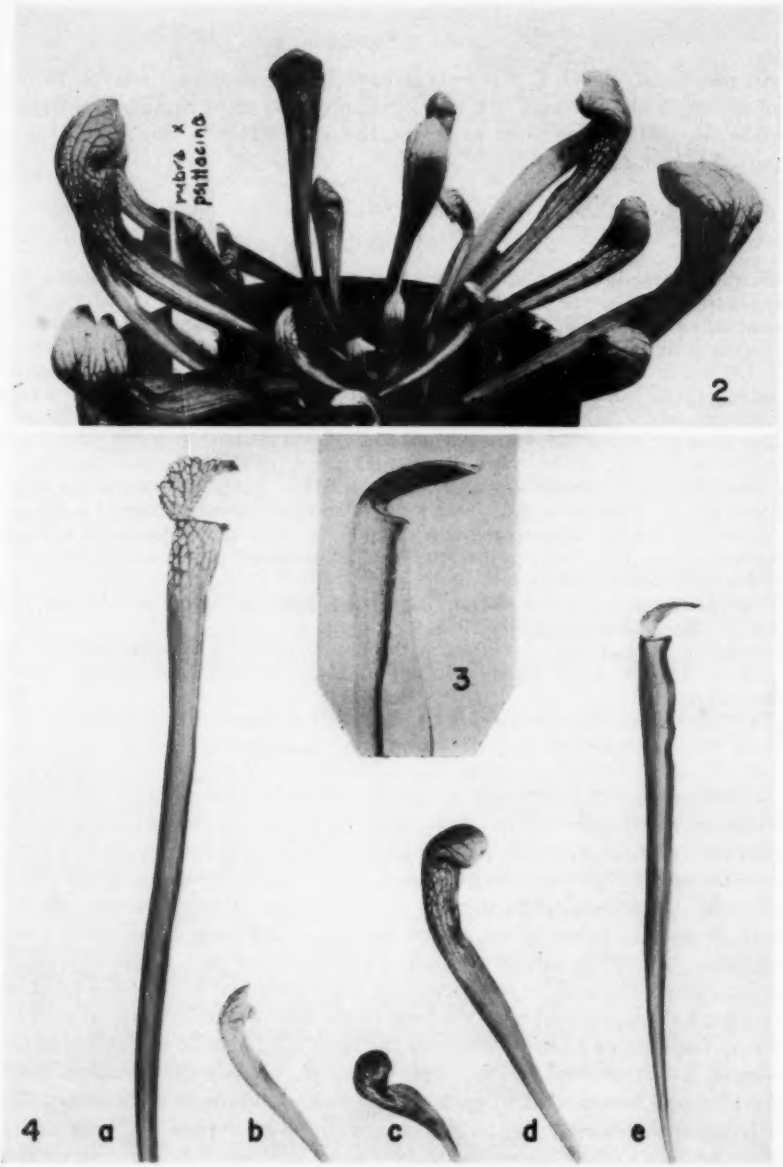

FIG. 2. *Sarracenia* ×*Gilpini*, ×⅓. FIG. 3. (*S. psittacina* × *rubra*) × *rubra*, upper portion of leaf, *ca.* ×½ FIG. 4. Leaves of: a, *S. leucophylla;* b, *S.* ×*Wrigleyana;* c, *S. psittacina;* d, *S.* ×*Gilpini;* e, *S. rubra;* all ×⅓, but 2 inches cut from bottom of the *S. leucophylla* leaf.

have scabrous adaxial surfaces and are therefore somewhat atypical. The anomalous condition of these hybrids can be explained as the result of an anomalous local condition of one of the parents and in no way refutes the field experience of the authors or the findings of Russell (1919) as regards the truly intermediate nature of *Sarracenia* hybrids. The dimorphic leaf condition, mentioned previously for another hybrid involving *S. psittacina* (Fig. 1), has also become evident in this hybrid under cultivation.

Four other plants from the type locality of this hybrid appear to be either backcrosses or F_2 segregates (Fig. 3). In view of the uniformity of these plants and their proximity to each other in the field the former interpretation of these plants seems more likely, and the plants have tentatively been identified (by leaf shape) as (*S. rubra* × *psittacina*) × *rubra*.

17. *S. alata* × *rubra*

Sarracenia ×Ahlesii hybr. nov.

Sarracenia ×Ahlesii, hybrida naturalis nova inter S. alatam et S. rubram, foliis erectis tubulatis e basi ad foramen gradatim accrescentibus, 1.2–4.5 dm. altis cucullo suberecto acuminato, floribus odoriferis et rubriluteis.

Plants intermediate between parents. Leaves erect, 1.2–4.5 dm. tall, narrowly tapered from base to orifice, green or yellow-green, the upper portions marked or shaded with maroon, adaxial wing narrow, linear, broadest below the middle; hood sub-erect 1.5–3.5 cm. broad, ovate-acuminate, adaxial surface puberulent, abaxial surface glabrous. Scape equalling or exceeding the height of the leaves. Flowers intermediate in size (to 7.5 cm. across when pressed), faintly sweet-scented; sepals ovate, slightly reflexed, green, shaded with maroon; petals strongly obovate, yellow, with maroon shading prominent on outer surface, inner surface yellow-green; style disc 2.0–2.5 cm. in diameter.

TYPE LOCALITY: Wet meadow 5 miles southeast of Fruitdale, Washington Co., Ala. 1955.

POSSIBLE RANGE: In association with parent species in limited areas of southwestern Alabama and southeastern Mississippi near the Gulf coast.

SPECIMEN EXAMINED: Alabama. Fruitdale, Washington Co., *Bell 1495* (NCU: type).

Another newly discovered natural hybrid, *Sarracenia ×Ahlesii*, was collected for the first time north of Deer Park, Alabama, in April, 1955. The plants were in bloom and were identified immediately by floral characteristics. Because *S. alata* is the only yellow-flowered species of *Sarracenia* in that region the yellow coloration in the petals presumably came from this species—this hypothesis was borne out later by leaf morphology. The maroon coloration in the flowers, coupled with the faint, sweet scent, the strongly obovate petals and the puberulent leaf hood could suggest no other red-flowered species than *S. rubra*, although *S. leucophylla* and *S. psittacina* were also at this locality.

A collection (*Bell 1456a*) from a large bog 5 miles south of Agricola, George county, Mississippi, has been tentatively referred to this category. The collection in question is from a single clump which had 18 beautiful, large, yellow-and-maroon flowers open at one time. The flowers are almost twice the size of the ones described above for *S. ×Ahlesii*, do not have the extremely obovate petals

and also lack the faint scent of this hybrid. However, the leaves show no trace of the form or hood pubescence to be expected if *S. purpurea* (the only other likely possibility at this location) were involved. This plant may be *S. alata* × *rubra*, as the leaves indicate, or it may be a segregate or second backcross (to *S. alata*) of *S.* ×*exornata*, as would be surmised from the flower.

18. *S. flava* × *psittacina*

Still unreported either as a natural or as an artificial hybrid, even though an intensive search was made for it at several promising localities in southern Georgia and northern Florida where the two potential parent species grow in the same areas.

19. *S. alata* × *psittacina*

POSSIBLE RANGE: In association with parent species in the limited range of southwestern Alabama (generally on a line west of Mobile) into southeastern Mississippi near the Gulf coast.

Several stations for plants presumed to be *Sarracenia alata* × *psittacina* have been found in the vicinity of Fowl River, Mobile county, Alabama. Neither the first collection (*Case P-62a*) nor a later one (*Bell 1534*) have bloomed, and none of the plants observed at other stations have shown any evidence of flowering. Because of the difficulty of distinguishing between the various hybrids of *S. psittacina* when in the vegetative stage (see No. 4, *S.* ×*Courtii*), the actual description of this hybrid must await the time when flowering material is available. Until such time there is a slight possibility that the collections cited above might be *S. purpurea* × *psittacina*, which is so far unknown as a natural hybrid.

SUMMARY

1. Two natural *Sarracenia* hybrids, *S.* ×*Gilpini* and *S.* ×*Ahlesii*, are reported, named, and described for the first time.

2. Collections of flowering material, previously unknown, of two other natural *Sarracenia* hybrids are reported and described.

3. Range extensions (new state records) are reported for four *Sarracenia* hybrids previously known.

4. Additional field data are given concerning most of the known natural hybrids in the genus *Sarracenia*.

LITERATURE CITED

BELL, C. R. 1952. Natural hybrids in the genus *Sarracenia*. I. History, distribution and taxonomy. Jour. Elisha Mitchell Sci. Soc. **68:** 55–80.

———. 1954. *Sarracenia leucophylla* Rafinesque. Jour. Elisha Mitchell Sci. Soc. **70:** 57–60.

HARPER, R. M. 1918. The American pitcher plants. Jour. Elisha Mitchell Sci. Soc. **34:** 110–125.

LAWRENCE, G. H. M. 1951. Taxonomy of Vascular Plants. Macmillan: New York.

MERRILL, ELMER D. 1948. Unlisted new names in Alphonso Wood's publications. Rhodora **50:** 101–130.

RUSSELL, A. M. 1919. The macroscopic and microscopic structure of some hybrid Sarracenias compared with that of their parents. Contr. Bot. Lab. Univ. Penn. No. **5:** 3–41.

THE NATURE OF TROPISMS OF *COPRINUS STERQUILINUS*[1]

By Donald B. Jeffreys[2] and Victor A. Greulach[3]

Introduction.—Little has been done to determine whether tropisms of fungi have the same physiological basis as those of vascular plants. Branbury (1952) found that *Phycomyces* sporangiophores did not react to unilateral applications of indole-3-acetic acid in any consistent manner, but they consistently exhibited negative curvatures with griseofulvin, a substance isolated by Oxford, Raistrick, and Simonart (1939) from *Penicillium griseofulvin*. Numerous investigators have studied the effect of auxin on mycelial growth. Leonian and Lilly (1937), Woif (1937), Murdia (1939), and Bhargava (1946) have reported that most of the fungi studied are indifferent to very low auxin concentrations but are inhibited by concentrations greater than a few parts per million. Kerl (1937), Richards (1949), and Fraser (1953) have reported that certain fungi are stimulated by auxin. Nielson (1932), Bonner (1932), Thimann and Dolk (1933), and Kogl and Verkaaik (1944) have shown that various fungi synthesize indole-3-acetic acid (IAA). The effect produced by IAA on mycelial growth provides no information concerning the tropisms of Basidiomycetes or other fungi. Streeter (1909) studied the geotropic responses of *Amanita phalloides* and *A. crenulata*. Hawker (1950) reported that a curvature in the stipe of *Collybia velutipes* could be obtained by removing one half of the pileus and also by placing a block of "fruit body extract agar" asymmetrically on the decapitated stipe.

In view of the scanty experimental work on the mechanism of fungal tropisms, this study was made to determine whether or not the geotropic and phototropic responses of *Coprinus sterquilinus* involve an auxin mechanism similar to that of higher plants. *C. sterquilinus* has been grown in pure culture by Buller (1931), who observed that the stipe was slightly phototropic. He found that light has an inhibitory effect on the base of the sporophores and on the sporophore primordia less than 1 mm. in diameter.

Methods.—The cultures were propagated from spores of a single pileus collected in 1951 near Chapel Hill, N. C. and were maintained on sterilized cow dung. Glass jars 8.5 cm. wide and 9 cm. high were two-thirds filled with dung, the top of each was covered with cotton, and then with paper, and the jars were autoclaved for 40 minutes at 120°C. Inoculations were made either by shaking up spores in sterile water and pouring it over the dung or by transferring a bit of mycelium from another jar. The cultures proceeded through several stages of development as described by Buller (1924, 1931) and after 15–17 days, mature sporophores were obtained. The height of these sporophores was 10–25 cm. Development was slow during the button stage, but after a height of 63 mm. had

[1] This paper is based on a master's thesis prepared by the author under the direction of the co-author. The authors wish to thank Dr. John N. Couch for suggesting the problem and for his advice during the course of the work.

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been reached rapid elongation of the stipe commenced and continued until maturity. The rapid elongation took 26–28 hours, and during this interval the length of the stipe increased 180–380 per cent. During this period the growth rate was quite uniform and when plotted described nearly a straight line. All of the experiments were performed while the stipe was elongating rapidly. Sporophores under observation were kept under bell jars when not actually being handled or measured.

A preliminary experiment indicated that the elongation of a *Coprinus* sporophore is similar to that of *Amanita*, as reported by Streeter (1909), in that in early stages the entire stipe elongates and in later stages the region of elongation gets successively smaller so that the lower part of the stipe ceases growing first and the region immediately below the pileus ceases growing last. In another preliminary experiment it was found that decapitations varying from the upper 5 mm. to the upper half of the stipe had no effect on the growth rate of the remaining part of the stipe.

The investigation consisted of three main groups of experiments: (1) an experiment concerned with phototropism, (2) three experiments concerned with geotropism and (3) three experiments concerned with unilateral treatments. In the phototropic experiment, a box measuring 17″ x 17″ x 30″, painted flat black, and with a circular opening 6.25 cm. in diameter at one end, was used. A light bulb connected to a rheostat was placed a few inches from the opening, and a rectangular battery jar filled with water was placed between the opening and the light. The *Coprinus* plants in the box were kept under bell jars. In one series, sporophores at an early stage of elongation, with the pileus covering nearly all of the stipe, were illuminated unilaterally. In the remaining two series the pilei were removed from all the sporophores. In one of these the entire stipe was illuminated unilaterally, and in the other series only the upper part of the stipe was illuminated. The curvatures were detected by superimposing photographs taken before and after exposure of the stipes to the light.

In the first geotropic experiment the geotropic responses of eight stipes with the upper fourth removed and eight stipes with the upper half removed were determined. After removal of the upper part of each stipe the remaining portion was placed in a horizontal position. To determine whether geotropic bending occurs in the region of the stipe which has stopped growing, the three stipes in the third geotropic experiment were marked at 4-mm. intervals, the rate at which the height of each mark was increasing was determined, and when the height of the lower marks had ceased increasing, the stipes were placed in a horizontal position, after which the region of curvature was determined. Curvatures in these experiments were measured with a protractor.

The purpose of the unilateral treatments was to determine whether or not the given treatment would induce a curvature. These experiments were performed in a darkroom. Illumination for working purposes was provided by a light passing through a Corning filter No. 2030, which has a cut-off at 6440 Å. The first series of unilateral treatments consisted of removing half of the pileus and a longitudinal half of the upper fifth of the stipe from the six experimental plants, which

FIGS. 1–6. Tropisms of *Coprinus sterquilinus*. FIG. 1. Geotropic response of sporophore which has been placed in a horizontal position after removing pileus and upper fourth of stipe. ×0.85. FIG. 2. Geotropic response after removing pileus and upper half of stipe. ×1.2. FIG. 3. Geotropic response of sporophore seven hours after placing in a horizontal position with pileus removed and stipe split into longitudinal sections. ×1. FIG. 4. Sporophore several hours after unilateral decapitation of pileus and upper fifth of stipe. ×0.88. FIG. 5. Superimposed photographs of sporophore showing results of unilateral illumination of upper half of stipe. Slight positive curvature is located in upper half. Second exposure was made six hours after first. ×0.65. FIG. 6. Superimposed photographs of sporophore showing results of unilateral illumination of the entire stipe. Note that entire stipe is involved in lateral displacement. Second exposure was made two hours after first. ×1.

were then maintained in an upright position. In the second series, stipes with the upper fifth decapitated were treated by placing variously prepared agar blocks unilaterally on the cut surface of the stump. The diffusate and the extract of *Coprinus* stipes, and different concentrations of indole-3-acetic acid (IAA), α-naphthalene acetic acid (NAA) and griseofulvin were incorporated into the agar blocks. Controls consisted of plain agar blocks. In the final series, stipes with the pilei removed were sprayed unilaterally with aqueous solutions of the following: 0.001, 1, 10, 50, 250 and 1000 parts per million NAA; 950 p.p.m. IAA; 50 p.p.m. griseofulvin; 0.5, 75, 100 and 500 p.p.m. 2,3,5-triiodobenzoic acid (TIBA); a mixture of 0.1 p.p.m. NAA and 0.25 p.p.m. TIBA; distilled water; and an extract made from *Coprinus* stipes.

RESULTS.—In the phototropic experiment no curvatures were detected among the young sporophores which had the stipes shaded by the pilei, indicating that the pileus is not sensitive to light. All the sporophores without pilei of which the entire stipes were illuminated exhibited positive phototropic curvatures, the amount of the stipe involved in lateral displacement varying according to age of the sporophore from 100 per cent down to only the upper 29 per cent. Among the 17 test plants of which only the upper part of the stipe was illuminated 12 exhibited a positive phototropic response restricted to the illuminated region (FIGS. 5, 6). Five sporophores had an unexplained negative response of around 10°, this bending occurring throughout the stipe.

Removal of the upper part of the stipe had no effect on the geotropic response of the lower part of the stipe (Figs. 1, 2). Splitting the stipe longitudinally did not interfere with geotropic bending, the sections of all of the stipes having a negative geotropic response of 90° or more (Fig. 3). Among the stipes which had been marked at 4-mm. intervals there were no geotropic curvatures in parts of the stipes which had ceased elongating.

Unilateral decapitation of the pileus and the upper fifth of the stipe did not cause any significant curvatures (Fig. 4). There were no significant curvatures among the 63 plants to which growth substances in agar blocks were applied. In at least 28 cases there was a slight lateral displacement of less than 5°, but these were about half positive and half negative and were obviously not significant. High concentrations of NAA, IAA and TIBA sprayed unilaterally on the stipes caused positive curvatures of significant magnitude, 40° with NAA, 20° with IAA and 23° with TIBA. Low concentrations of these substances had the same effect as distilled water, as did the extract of *Coprinus* stipes. Of six sporophores sprayed with 50 p.p.m. griseofulvin three had no curvatures and three had negative curvatures which averaged 10°. Sporophores sprayed with distilled water responded in a definite pattern. After 20 minutes a negative curvature of about 5° could be detected. The stipe would then gradually return to the vertical, and after an hour or two a positive curvature of as much as 5° was frequently present. Spraying a second time was sometimes necessary to induce this positive curvature.

DISCUSSION.—The mechanism governing tropisms of *C. sterquilinus* appears to be different from that of vascular plants. The results of the experiments were

in direct contrast to what would have been expected had a vascular plant such as *Avena* been the test organism. Decapitation did not reduce the growth rate, phototropic bending was restricted to the illuminated region of the stipe, the geotropic response of decapitated stipes was comparable to that of similar regions of untreated controls, unilateral decapitations did not cause bending and auxins did not cause negative curvatures. Failure of decapitation to interfere with the growth rate or geotropic response indicates that growth in the lower part of the stipe is not governed by a hormone transported from the upper portion. Physiological regeneration of the tip does not appear to be a likely explanation since there was no temporary growth inhibition following decapitation.

Failure of the agar block technique to evoke either positive or negative curvatures of significance, whereas spraying with high concentrations of auxins did cause marked positive curvatures, is an indication that the substance in the block is not translocated longitudinally through the stipe. Since none of the growth substances applied unilaterally to stipes caused significant negative curvatures, it seems evident that those substances which normally stimulate growth in vascular plants do not have a comparable effect on *C. sterquilinus*. The positive curvatures obtained by spraying with high concentrations of auxin indicate inhibition of growth. High concentrations of auxin also inhibit growth in vascular plants, but the mechanism of this inhibition is not well understood. It should be noted that TIBA is an anti-auxin, yet it had the same effect as IAA and NAA in that it inhibited growth. It seems quite possible that the inhibition may have been due to some factor other than a specific auxin action.

In view of the basic differences between vascular plants and Basidiomycete sporophores as regards structure, cell wall composition, tissue differentiation and physiological differentiation, it would not be surprising to find a difference in the systems governing their tropisms. The results suggest, not only that auxin is not involved, but also that no other hormone is involved. It seems likely that each hyphal strand responds individually to environmental factors. Because the strands are aggregated, this results in a unit action by the stipe.

SUMMARY

Removing the upper half or upper fourth of the stipes of the mushroom *Coprinus sterquilinus* did not affect the growth rate or the geotropic response of the stump. When the stipe was split into separate longitudinal sections, each section independently exhibited a geotropic response. Illumination of the upper part of the stipe while the lower part was shaded caused phototropic bending in the illuminated part only. No significant curvatures were obtained by decapitating stipes and placing unilaterally on the stump agar blocks containing growth substances, diffusate of *Coprinus sterquilinus* or diffusate of *Avena* coleoptiles. The spraying of high concentrations of growth substances on one side of the stipe caused significant positive curvatures, but the effect of low concentrations did not differ from the effect of distilled water. It is concluded that the system governing tropisms in *Coprinus sterquilinus* differs from that of vascular plants.

LITERATURE CITED

BHARGAVA, K. S. 1946. Physiological studies on some members of the family Saprolegniaceae. V. Growth substances. Lloydia **9:** 13–23.

BONNER, J. 1932. The production of growth substance by *Rhizopus suinus*. Biol. Zentralbl. **52:** 565–582.

BRANBURY, G. H. 1952. Physiological studies in the Mucorales. Part II. Some observations on growth regulation in the sporangiophore of *Phycomyces*. Jour. Exper. Bot. **3:** 86–94.

BULLER, A. H. R. 1924. Researches on Fungi. III. Longmans, Green and Co.

———. 1931. Researches on Fungi. IV. Longmans, Green and Co.

FRASER, I. M. 1953. The growth promoting effect of indole-3-acetic acid on the common cultivated mushroom *Psalliota hortensis* (Cooke). Austral. Jour. Biol. Sci. **6:** 379–395.

HAWKER, L. E. 1950. Physiology of Fungi. University of London Press.

KERL, I. 1937. Uber Regenerationsversuche an Fruchtkopern und anderne entwicklungsphysiologische Untersuchungen bei *Pyronema confluens*. Zeitschr. f. Bot. **31:** 129.

KOGL, FRITZ UND BERNARD VERKAAIK. 1944. Uber das Vorkommen von Auxin in lichtempfindlichen Pilzen. XXXVII. Uber pflanzliche Wachstumsstoffe. Hoppe-Seyler's Zeitschr. Physiol. Chem. **280:** 162–166.

LEONIAN, L. H. AND V. G. LILLY. 1937. Is heteroauxin a growth promoting substance? Amer. Jour. Bot. **24:** 135.

MURDIA, M. S. 1939. Effect of beta-indole-3-acetic acid and phenyl acetic acid on the growth of some members of the family Saprolegniaceae. Cur. Sci. **8:** 362–363.

NIELSEN, N. 1932. Uber das Vorkommen von Wuchsstoff bei *Boletus edulis*. Biochem. Zeitschr. **249:** 196–198.

OXFORD, A. E., RAISTRICK, H., AND P. SIMONART. 1939. Studies in the biochemistry of micro-organisms. LX. Griseofulvin, $C_{17}H_{17}O_6Cl$, a metabolic product of *Penicillium griseofulvin* Dierckx. Biochem. Jour. **23:** 240–248.

RICHARDS, REBEKAH RUTH. 1949. Responses of representative fungi to certain growth regulating substances. Bot. Gaz. **110:** 523–550.

STREETER, STELLA G. 1909. The influence of gravity on the direction of growth of *Amanita*. Bot. Gaz. **48:** 414–426.

THIMANN, K. V. AND H. E. DOLK. 1933. Conditions governing the production of plant growth hormones by *Rhizopus* cultures. Biol. Zentralbl. **53:** 49–66.

WOLF, F. T. 1937. A nutritional study of *Achlya bisexualis* and *Saprolegnia ferax*. Amer. Jour. Bot. **24:** 119–123.

MAINTENANCE OF STOCK CULTURES OF AQUATIC FUNGI

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In most laboratories where a variety of aquatic fungi are grown for teaching and research, time and help are too short for the classical method of maintaining the stock on agar slants with frequent routine transfers. Furthermore, although most watermolds lend themselves readily to this method, many are easily "lost," and some aquatic fungi cannot be maintained on agar at all. Over many

years, observations have been made in this laboratory on other simple methods, and these have been studied more intensively by the writer since 1948. Some of the records presented here, although often incomplete and lacking in replication, are of considerable interest. The writer is grateful to Dr. John N. Couch for inspiration and direction, and for certain cultures. Cultures were received also from Dr. A. W. Ziegler and Mr. W. R. Pendergrass of this laboratory; Mrs. Helen S. Reischer; the American Type Culture Collection; and the Centraalbureau voor Schimmelcultures.

PETRI DISHES. (A) *Wet.*—A stock of cultures on the laboratory table can be kept going almost indefinitely by the periodical addition of fresh water and bait. Unsatisfactory results with distilled or tap water may be improved by treatment with charcoal (Couch, 1927). Dilute soilwater[1] usually encourages vigorous growth, but also promotes contaminants, and is therefore unsuitable for cultures to be kept long in Petri dishes. Hemp seeds have been used as standard bait successfully in this laboratory since 1923 for most watermolds. The seeds are sterilized by boiling for about five minutes, since autoclaving renders them too soft, and are then halved with a sterile knife. Grass, fruits, twigs, insects, hair, feathers, etc. provide a more suitable substratum for certain other fungi. Growth of algae and infestation by mites can be controlled by placing the dishes in a dark, alcohol-swabbed container. A cool temperature will reduce the frequency of attention required. Removal from sight, however, increases the likelihood of neglect and loss of cultures by drying up.

(B) *Dry.*—Intentional drying of members of the Blastocladiaceae and some of their parasites, as a method of preparation for storage, has met with staggering success. *Allomyces arbusculus*, *A. anomalus*, *A. moniliformis*, *A. macrogynus* (previously called *A. javanicus* var. *macrogynus*) and two strains of *A.* ×*javanicus* (previously *A. javanicus*)[2] have all been kept dry for months or years. Most of the water is poured off sporophytic hemp seed cultures that are at least three weeks old, and then the dish is inverted, allowing the rest of the water to dry off gradually. Cultures of *A. moniliformis* were dried in this way in May, 1951 and revived by the addition of fresh water and hemp seeds in July, 1954—more than three years later. Occasionally, however, a culture fails to revive after this treatment, when dried on the glass of the dish. Complete success is achieved by allowing each culture to dry on a piece of sterile filter paper about an inch square. Several squares can then be stored together in a labelled, dated envelope, requiring no attention and very little space for years. Thirty-seven cultures of *A. arbusculus* which had been collected and prepared for storage in this way between 1936 and 1939, by a number of investigators in this laboratory, were tested for survival in the summer of 1954. All revived except two, one of which seemed to have been eaten by carpet beetles. These cultures, therefore, had remained viable

[1] 500 gm. garden soil in 1000 ml. water, heated for 2 hrs. in a waterbath; filtered, and restored to 1000 ml.; autoclaved 15 mins. at 15 lbs. pressure. Dilutions of 1/10 to 1/20 usually best for growth. (Adapted from Bold, 1942.)

[2] For the redesignation of certain species of *Allomyces* see Emerson and Wilson, Mycologia **46**: p. 429, 1954.

for more than 14 years, and some of them for 17 years. The parasites, *Catenaria allomycis* and *Rozella allomycis*, can also be kept in this way, on dried cultures of their hosts.

Blastocladiella cystogena can be stored in the above manner if pieces of grass (*Paspalum*) are used as substratum. Six pieces, dried in August, 1942 and wrapped and stored in separate envelopes, were tested in November, 1954. Abundant new growth was obtained from all six. These cultures, therefore, had remained viable for more than 12 years.

Among other groups, many species of *Pythium* can be recovered from dried cultures; but only certain members of the Saprolegniaceae can be counted on to survive, and the method cannot be relied upon for them.

FLASKS.—Plugged Erlenmeyer flasks, although bulky, will reduce the contamination and drying-out encountered with Petri dishes; straight-sided bottles with screw-caps would be better. The increased depth of water also helps to keep the respiration rate low. The beneficial effect of leaves, especially oak, in promoting healthy growth of watermolds, has long been recognized in this laboratory. On June 29, 1949, the following experiment was set up: Pieces of oak and beech leaves about two inches square were sterilized in water in a large flask. Then 8 sets of 125-ml. flasks were prepared, each of the first 6 containing 30 ml. of charcoal-treated tap water or leaf solution: (1) with a piece of oak or beech leaf in the solution resulting from its sterilization; (2) as above, but with the solution poured off and replaced by water; (3) with leaf solution only; (4) with a piece of filter paper in leaf solution; (5) with a piece of filter paper in water; (6) with water alone; (7) with about 2 teaspoonfuls of soil and 10 ml. water; and (8) with a small amount of *Sphagnum* moss and 10 ml. water. The flasks were inoculated in duplicate with hemp seed cultures of *Saprolegnia ferax*, *Achlya flagellata*, *Isoachlya unispora*, *Dictyuchus monosporus* and *Thraustotheca primoachlya*. On October 16, all the soil and all the moss cultures were dry, and the only watermold recovered from them was *D. monosporus*, from the moss. Among the rest, all were recovered except *I. unispora* from the flasks with the leaf in water and the leaf in its own solution, and *D. monosporus* from the leaf in its own solution; in all three, the leaf used was oak. Sterilization of the oak leaves gave a much stronger, darker-colored solution than of the beech leaves. In this experiment, it was clear that sterile water was adequate, for four months. More difference might have been apparent among the sets if all the flasks had been kept long enough to dry, but contamination by *Aspergillus* and *Penicillium* was already a problem.

On Dec. 20, 1951, three sets of flasks were prepared containing distilled water, dilute soilwater, or dilute oak-leaf solution. Into these were placed, in duplicate, hemp seed cultures of *Saprolegnia ferax*, *Achlya radiosa*, *Achlya* sp. (9F), *Isoachlya intermedia* and *Brevilegnia linearis*. The flasks remained in the laboratory until Feb. 1, 1952, when they were transferred to the cold room at 10°C. They were tested for survival in July, 1954. From the distilled water, all isolates were recovered except a duplicate of each of *I. intermedia* and *B. linearis*, which were badly contaminated; from the soilwater, all were recovered except duplicates of

A. radiosa and *B. linearis* (this was badly contaminated); none were recovered from the oak-leaf solution. Again, sterile water had been adequate, this time for 31 months. The oak-leaf solution was probably still too strong, and was definitely harmful.

In later work, the leaves were sterilized at least three times before inoculation, and the leaf solution was poured off and replaced by sterile water. Some results are recorded in Table I, which shows that a number of species were recoverable after more than 18 months. Some of these were completely dry. In addition, nine sterile strains of *Dictyuchus* not included in the table, which were in the cold room in flasks with pieces of oak leaves, were all recovered 18 months later.

TEST TUBES. (A) *Water.*—It was found that two members of the Plasmodiophoraceae which occur as parasites of *Pythium*, *Sorodiscus Cokeri* (three strains) and an undescribed species of *Woronina* (three strains), were frequently lost when their hosts were allowed to dry, although the hosts themselves might be recoverable. They will not grow well, if at all, in agar cultures. They can, however, be kept very conveniently in test tubes about one-third full of water, in hemp seed cultures of their hosts. For example, *S. Cokeri* survived in this way for 31 months at 18°C. For these organisms, tubes containing sterile soil seemed to offer no advantage over those containing water only. If either dried out, the parasite was not often recoverable by the usual simple methods. This does not necessarily mean that the resting spores were no longer viable, for the conditions required for their germination are not well understood in the Plasmodiophoraceae.

On the other hand, the Actinomycete genus, *Actinoplanes*, is readily recoverable from dried soil. About 150 isolates have been inoculated into soil about 2 cm. deep in screw-cap test tubes, 125 x 20 mm., to form an accessory collection to the regular stock on agar. The garden soil was autoclaved twice in shallow trays before its final sterilization in test tubes with enough water to moisten but not water-log it. To recover the organism, the soil is transferred to Petri dishes, sterile water is added, and it is baited with *Liquidambar* pollen and pieces of boiled *Paspalum* leaves. So far, only eight isolates have been tested, five months after inoculation, and all were recovered. For such a notoriously variable group of organisms, this method has the particular advantage over agar cultures, which require repeated transfers, of depressing growth over long periods of time and hence of keeping variation to a minimum. Furthermore, despite its sterilization, the soil is a comparatively "natural" medium.

(B) *Agar.*—Cultures on agar must be absolutely pure. Watermolds can be cleaned by Raper's (1937) method, by which aerobic contaminating organisms are held within a sterile glass ring while the mycelium of the mold grows below and beyond it; unless the Petri dishes are unusually flat, it is not generally necessary to raise the ring on "feet." Before regular test tubes were replaced by screw-cap tubes, many cultures were lost, often after only a few months, as a result of the growth of contaminants in the plug (see Table I). The following records for storage on Difco cornmeal agar slants at 10°C., not all included in the table, are notable: *Isoachlya unispora* and *Thraustotheca clavata*, 16 months; *Dictyuchus monosporus*, 17 months; *Achlya racemosa*, 19½ months (cf. Table I);

TABLE I

Survival of Saprolegniaceae on hemp seeds in water, with and without pieces of leaf, and on cornmeal agar slants

In each of the four columns are given: month of inoculation; number of months until test; result, those recovered being marked +, not recovered, −. (The Roman numerals indicate different strains. O, oak leaf; B, beech leaf.)

	Laboratory. Fluctuating temperature				Cold room. 10°C.		
	125-ml. Erlenmeyer flasks with 30 ml. water						Cornmeal agar slants
	Water only		Water + leaf		Water + oak leaf		
A. americana I	June 4 mo. +		(O) Oct. 6½ mo. −		Apr. 19 mo. −		May 4½ mo. +
A. americana II	July 3 mo. +		(O) Oct. 4 mo. +		May 18 mo. +		July 8 mo. +
A. apiculata	June 4 mo. −		(B) Nov. 6 mo. −				June 4 mo. −
A. caroliniana	May 4½ mo. −		(B) Oct. 6½ mo. +				May 4½ mo. +
A. colorata	May 4½ mo. −		(B) Nov. 6 mo. −				May 4½ mo. +
A. crenulata					Apr. 19 mo. +		June 4 mo. −
A. dubia			(O) Oct. 6½ mo. +		May 17 mo. +		
A. flagellata	May 4½ mo. +		(O) Oct. 6½ mo. −		Apr. 19 mo. +		May 4½ mo. −
A. glomerata	June 3½ mo. +		(B) Oct. 6½ mo. −		Apr. 12 mo. +		June 4 mo. −
A. imperfecta	May 4½ mo. +		(O) Oct. 6½ mo. −		Apr. 19 mo. +		May 10 mo. +
A. klebsiana	June 3½ mo. +		(O) Oct. 7½ mo. +		May 17 mo. +		June 3½ mo. −
A. racemosa	June 3½ mo. +						May 3½ mo. −
S. feraz	May 4½ mo. +		(O) Oct. 6½ mo. +		Apr. 19 mo. −		June 4 mo. −
S. diclina I	June 4½ mo. +		(O) Oct. 6½ mo. −		Apr. 19 mo. −		June 4 mo. +
S. diclina II	May 4½ mo. −		(B) Nov. 5½ mo. +		Apr. 19 mo. +		May 4½ mo. +
S. litoralis	June 3½ mo. +		(O) Oct. 6½ mo. +		Apr. 18 mo. +		June 4½ mo. +
S. monoica	June 3½ mo. +		(O) Oct. 6½ mo. −		Apr. 18 mo. +		
S. monoica var. *glomerata*	May 4½ mo. +		(O) Oct. 6½ mo. +		Apr. 19 mo. +		Oct. 4½ mo. +
I. unispora			(O) Oct. 6½ mo. +		Apr. 19 mo. +		May 16 mo. +
T. clavata			(O) Oct. 5½ mo. +		Apr. 19 mo. +		May 16 mo. +
T. primoachlya	May 4½ mo. +		(O) Oct. 17½ mo. +		Apr. 18 mo. +		June 4 mo. −
D. monosporus	June 3½ mo. +		(O) Oct. 17½ mo. +		Apr. 18 mo. +		Apr. 17 mo. +

Pythium catenulatum, *P. irregulare*, *P. proliferum* and *P. undulatum*, 19½ months. In general, species of *Pythium* are more hardy than the Saprolegniaceae under this treatment, and will often remain viable until the agar has dried hard. Table II shows the viability of five species of Saprolegniaceae after at least 28 months on three different agars at 10°C. and 18°C. Maltose-peptone agar (3 gm. maltose; 1 gm. peptone; 20 gm. agar; 1000 ml. water) was the least favorable; Difco cornmeal agar (19 gm. agar preparation; 1000 ml. water) and potato dextrose agar (300 gm. diced potatoes boiled for an hour in a liter of water, filtered through a towel, restored to volume; 20 gm. dextrose; 20 gm. agar) were about equal. *Saprolegnia ferax* and *Isoachlya intermedia* were outstandingly resistant; *Brevilegnia linearis* did not survive on any of the three agars alone, and was recovered only from the cornmeal agar submerged under mineral oil (see below). All the other four species survived on all three agars, at 18°C., when submerged under oil.

(C) *Agar under mineral oil.*—The metabolic rate of the fungus, as well as the drying-out of the agar, can be depressed to a minimum by submerging the agar cultures under heavy mineral oil, as described by Buell and Weston (1947). This was shown by Reischer (1949) to be highly successful for a large number of isolates of Saprolegniaceae, some of which were kept submerged for as long as 30 months. The results shown here in Table II give eloquent testimony to the value of the method.

In June, 1949, *Saprolegnia ferax*, *Achlya flagellata*, *Isoachlya unispora*, *Dictyuchus monosporus* and *Thraustotheca primoachlya* were separately inoculated in octuplicate on slants of each of three agars: Difco cornmeal, oatmeal (2 heaped tablespoonfuls oats boiled for an hour in a liter of water, squeezed through a towel, restored to a liter; 20 gm. agar), and maltose-peptone. Five days after inoculation, four tubes of each isolate on each agar were sealed with parafilm; two were placed in the cold room at 10°C. and two in the laboratory. The other

TABLE II

Survival of Saprolegniaceae on three agars at 10°C. and 18°C., with and without mineral oil

Inoculated in Dec., 1951 and tested between March and July, 1954, i.e. after at least 28 months. Survival is shown by +; loss, by −.

Agar	Temp.	*S. ferax*	*A. radiosa*	*Achlya* sp.	*I. intermedia*	*B. linearis*
Cornmeal	10°C.	+	+	−	+	−
	18°C.	+	−	+	+	−
	18°C. (oil)	+	+	+	+	+
Potato-dextrose	10°C.	+	−	+	+	−
	18°C.	+	−	+	+	−
	18°C. (oil)	+	+	+	+	−
Maltose-peptone	10°C.	+	−	−	+	−
	18°C.	−	−	−	−	−
	18°C. (oil)	+	+	+	+	−

TABLE III

The longest period of survival, in years and months, of five members of the Saprolegniaceae in quadruplicate on three agars under mineral oil

Agar	*S. feraz*	*A. flagellata*	*I. unispora*	*D. monosporus*	*T. primoachlya*
Cornmeal.....	4 yr. 4 mo.	2 yr. 6 mo.	Not recovered	4 yr. 4 mo.	4 yr. 4 mo.
Oatmeal.....	5 yr. 1 mo.	5 yr. 1 mo.	2 yr. 6 mo.	5 yr. 1 mo.	5 yr. 1 mo.
Maltose-peptone.....	2 yr. 6 mo.	10 mo.	2 yr. 6 mo.	10 mo.	2 yr. 7 mo.

four cultures in each set were submerged under sterile mineral oil and stored in the laboratory. Tubes were removed for testing at various times up to July, 1954. The tests were carried out by transferring a portion of the agar containing mycelium to a Petri dish, draining off as much oil as possible, washing with a strong squirt of sterile water from a wash-bottle, and leaving it with a piece of hemp seed on top of it, either in water or in a liquid medium[3] used at the American Type Collection for "revitalizing sluggish cultures;" or by transferring it, with or without washing, to a dish of cornmeal agar, dragging it over the surface to free it partially from the oil clinging to it. By March, 1950, most of the unsubmerged cultures were useless on account of contaminants on the parafilm and in the plug, although all but *D. monosporus* were recovered from the maltose-peptone agar tubes in the cold room. Unaccountably, the plugs of the tubes containing oil were still clean, and the results with these are shown in Table III. Oatmeal agar, a rich medium, was clearly the best, four of the five species surviving on it for over five years; as in Table II, maltose-peptone agar was the least favorable.

Submergence under oil has been adopted here as a standard method for keeping stock cultures of watermolds, and especially Saprolegniaceae, although it may also be suited to certain members of Blastocladiaceae, Leptomitaceae, and Pythiaceae (Table IV). A species of *Rhizidiomyces*, in the Hyphochytriaceae, survived under oil for 19 months on three tubes of maltose-peptone agar (in two of these, the agar was half-strength). Because of its ease of preparation and general usefulness, Difco cornmeal agar has most often been used. Miscellaneous records obtained over a number of years are summarized in Table IV. Some of the results seem to be conflicting. This may be due, in part, as suggested by Mrs. Reischer for a similar conflict in her results, to the nutritional inadequacy of this medium for long periods of growth. *Isoachlya unispora*, for instance, survived on cornmeal agar alone for 16 months (Table I); under oil, it survived for 13 months but was lost before 33 months (Table IV); again, it survived for 30 months on oatmeal agar and maltose-peptone agar but was lost early from cornmeal agar (Table III). Sometimes, perhaps, the cultures were imperfectly cleaned. There are indications, however, of some individuality in the response of the different species. Thus, the method is clearly unsuited to *Achlya apiculata* (Table IV). *Achlya colorata*, *A. racemosa* (not recorded in the table) and *A. radiosa*, which

[3] Proteose peptone, 5 gm.; tryptose, 5 gm.; KH_2PO_4 0.2 gm.; water, 1000 ml. (Communication from Dr. Freeman A. Weiss, Oct. 6, 1952.)

TABLE IV

Survival of watermolds on cornmeal agar under mineral oil at fluctuating laboratory temperature

More than one number under "Period in months" indicates that more than one culture was investigated. Roman numerals indicate different strains.

Survived		Not recoverable by methods used	
Species	Period in months	Species	Period in months
Achlya americana I	54, 68	*Achlya americana* II	10, 30
A. americana II	20, 29	*A. apiculata* I	10, 23, 30
A. crenulata	9, 30, 30	*A. apiculata* II	23
A. dubia	43	*A. apiculata* III	17
A. flagellata I	32	*A. flagellata* I	60
A. flagellata II	9, 14, 32	*A. radiosa*	26
A. glomerata	9, 30	*Saprolegnia diclina* I	53
A. Klebsiana	26, 31, 42	*S. diclina* III	12
A. Orion	17	*S. diclina* IV	56
Achlya sp. (9F)	23	*S. monoica*	42
Saprolegnia ferax I	31, 33	*S. mixta*	22
S. ferax II	26, 31	*S. delica*	48
S. diclina I	19, 20, 26	*Isoachlya unispora*	33, 43, 62
S. diclina II	56	*I. intermedia*	22, 37
S. litoralis	10, 11, 30	*Thraustotheca clavata*	43
S. monoica	40	*Aphanomyces laevis*	41, 43, 46
S. monoica var. *glomerata*	43	*Brevilegnia linearis*	13, 19, 42, 50
S. mixta I	45		
S. mixta II	30, 37	*Leptolegnia subterranea*	39, 42, 63
S. delica	11	*Dictyuchus* sp. (4)	61
Isoachlya unispora	13	*Dictyuchus* sp. (11)	52
I. intermedia	20	*Dictyuchus* sp. (13)	52
Thraustotheca clavata	10, 46	*Dictyuchus* spp. (18–20, 22)	52
T. primoachlya	19		
Aphanomyces laevis	10	*Sapromyces androgynus*	34
Brevilegnia linearis	9		
Leptolegnia subterranea	9	*Allomyces anomalus*	36
Dictyuchus monosporus I	20, 50		
D. monosporus II	23, 52		
Dictyuchus sp. (3)	62		
Dictyuchus sp. (10)	34		
Dictyuchus sp. (12)	38		
Dictyuchus sp. (15)	43		
Dictyuchus sp. (21)	52		
Pythium catenulatum	41		
P. irregulare	43		
P. periplocum	43		
P. proliferum	29		
P. pulchrum	16		
P. vexans	37		
Apodachlya brachynema	34		
Allomyces moniliformis	34		

are apparently closely related, were all lost early in the investigation, and had to be replaced. *Aphanomyces laevis*, *Brevilegnia linearis*, and *Leptolegnia subterranea*, also, are undependable.

CONCLUSIONS

Different methods are suitable for different groups of organisms, to maintain them in culture for a long time without attention. For the organisms in this laboratory, the following are most successful: Blastocladiaceae and certain of their internal parasites, drying on filter paper; Saprolegniaceae, submergence under oil of cultures on a rich nutrient agar, or cool storage of hemp seed cultures in water with oak leaves (the solution resulting from the sterilization of the leaves in water, unless *very* dilute, is harmful and should be replaced by water); Pythiaceae, cool storage of agar cultures; Actinoplanaceae, drying in soil. A certain amount of individuality may be expected in the response of the various members of any one group, and for some of them no single method should be relied upon completely.

LITERATURE CITED

BOLD, H. C. 1942. The cultivation of algae. Bot. Rev. **8**: 69–139.

BUELL, CAROLINE B., AND WILLIAM H. WESTON. 1947. Application of the mineral oil conservation method to maintaining collections of fungous cultures. Amer. Jour. Bot. **34**: 555–561.

COUCH, J. N. 1927. Some new water fungi from the soil, with observations on spore formation. Jour. Elisha Mitchell Sci. Soc. **42**: 227–242.

RAPER, J. R. 1937. A method of freeing fungi from bacterial contamination. Science **85**: 342.

REISCHER, HELEN S. 1949. Preservation of the Saprolegniaceae by the mineral oil method. Mycologia **41**: 177–179.

SUGGESTIONS TO CONTRIBUTORS

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Department of Botany
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